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INFLAMMATION IN EMBRYONIC LIFE

I. CHANGES PRODUCED BY PARTICULATE MATTER AND BY A CHEMICAL AGENT *

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Although a great variety of infectious agents have been grown upon the membranes of chick embryos, little detailed information concerning the character of inflammation in embryonic life has been obtained. In the present study changes following the introduction of particulate matter and of a chemical irritant have been observed at different stages of development.

Bauer [1] applied different agents, including benzine, a mixture of benzine and paraffin, aniline, and aluminum powder, to the outer surface of the chorion by introducing it into the air space of the shell. These substances caused widely distributed rarefaction of the mesenchymal tissue in some places and hyperplasia elsewhere; formation of blood vessels and hemopoiesis were more active than usual. Local changes produced by these agents were not studied. Proliferative changes in the three layers of the chorioallantoic membrane following removal of a part of the eggshell have been described by Goldsworthy and Moppett.[2]

When Schneider [3] injected carbon particles of India ink in large quantity into the vitelline vein of chick embryos from 2 to 14 days old, they were found in endothelial cells in all parts of the embryo and its membranes, but when the quantity injected was diminished, endothelium of blood vessels of the area vasculosa, of the liver and of the glomeruli took up carbon particles whereas other endothelial cells contained little or none. At this stage of development no difference between Kupffer cells and other lining cells of the capillaries of the liver was evident.

The chorioallantoic membrane has been used by Goodpasture, Woodruff and Buddingh [4] for the cultivation of many filterable viruses.

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Woodruff and Goodpasture[5] infected with fowl pox the chorioallantoic membrane and embryonic skin of chick embryos at an early stage of development and found proliferation of ectodermal and of entodermal cells with inclusion bodies in both. Infection of chick embryos, 12 to 19 days old, with Rocky Mountain spotted fever by Lillie[6] caused infiltration of the membrane with cells resembling lymphocytes and proliferation of fibroblasts about blood vessels.

Cultures of *Staphylococcus aureus* and of hemolytic streptococci applied by Goodpasture and Anderson[7] to the chorioallantoic membrane of chick embryos from 6 to 14 days old caused superficial necrosis but these microorganisms did not invade the tissues of the membrane. Diphtheria bacilli grew upon the surface and apparently killed the embryo by their toxin. Typhoid bacilli entered the ectodermal cells of the membrane. *Brucella abortus* and the avian tubercle bacillus entered ectodermal and entodermal cells and, penetrating into the mesoderm, were found in fibroblasts and mononuclear phagocytes. Both epithelial and mesodermal cells were favorable sites for the multiplication of these bacteria.

Meningococci inoculated by Buddingh and Polk[8] upon the chorioallantoic membrane of chick embryos 12 days old invaded the blood vessels of the membrane and, widely distributed by the blood, found lodgment in the meninges, in the glomeruli of the kidneys and elsewhere. In the meninges they produced meningitis. In embryos 15 days old the microorganism proliferated more slowly, there was greater accumulation of polymorphonuclear leukocytes and no lesions of internal organs were found. Gonococci similarly introduced caused, Bang[9] found, clouding and ulceration of the membrane with local accumulation of polymorphonuclear leukocytes, but no invasion of internal organs.

The developing embryo was used by Rous and Murphy[10] for the study of tumor implantation. Murphy[11] showed that sarcoma and mammalian embryonic tissue implanted upon the membrane of chick embryos grew actively, whereas they failed to grow when grafted into an adult fowl. If the foreign tissue were implanted on the 19th or 20th day of incubation, that is, shortly before hatching, growth failed to occur, and if the tissue had already been implanted in the embryo, growth ceased at the same period of development, the graft being destroyed. In both cases active new formation of fibrous tissue occurred about the graft, and it was invaded and replaced. Danchakoff,[12] Minoura[13] and Huxley and Murray[14] have observed proliferative changes with cornification in the ectoderm of the chorioallantois produced by the presence of implanted tissue.

Methods

Chick embryos ranging in age from 36 hours up to the period of hatching received injections of India ink diluted to five times its volume with salt solution, or of turpentine which had been mixed with India ink to mark the site of injection.

Embryos younger than 12 days were injected through capillary pipettes directed by a micromanipulator and older embryos with a tuberculin syringe and fine needle. The shell at the summit of the large end of the egg, taken temporarily from the incubator, was removed with sterile instruments and the shell membrane was then torn away with fine forceps. When embryos were younger than 3 days, from 8 to 12 cc. of albumen were removed with a pipette so that the embryo resting on the top of the yolk could be reached more readily. The opening was covered with part of the shell of another egg and returned to the incubator.

Injections into the bodies of young embryos were made near the tail because those elsewhere have often caused death of the embryo. With older embryos it was possible by gentle manipulation to expose for injection a leg or wing. After different intervals following the injection of an irritant, embryos were removed from the egg and fixed in Bouin's solution. Eggs were opened but left uninjured otherwise, and were examined after incubation as controls. Small embryos or the injured parts of larger embryos were sectioned serially. Sections were stained with a variety of methods but the best results were obtained with hematoxylin and eosin-azure and by examination under the oil immersion lens.

Inflammation Caused by Carbon Particles

Changes in the Membranes

When a suspension of carbon was injected into the amniotic cavities of embryos 3 to 5 days old, usually with injury to the area pellucida close to the body of the embryo by the injecting instrument, the most conspicuous response was *accelerated proliferation of cells* at and near the site of injury. In the chorion and amnion there was hyperplasia of ectoderm and mesoderm and in some instances it had closed the wound. The hyperplastic ectoderm formed multiple superimposed cells, and beginning abnormal keratinization was shown by the deep eosin stain of the superficial cells (embryo no. 138, 4¾ days old, and examined 18 hours after injury). About a small collection of carbon particles proliferating ectodermal cells of the amnion formed a rounded projecting nodule.

The underlying mesodermal cells underwent similar multiplication,

and mitoses were numerous. When injected carbon particles accumulated in contact with the wall of a small blood vessel, proliferation of endothelial cells occurred and was limited to the part of the endothelium adjacent to the carbon so that a small mass of cells projected into the lumen (no. 39, 3½ days old, examined after 24 hours).

In an embryo (no. 37) injected when 3½ days old and fixed 10 hours later, numerous *mononuclear phagocytes* containing carbon particles were found in the cavity between chorion and amnion and in contact with the inner surface of the former. Some of the cells that ingested carbon contained erythrocytes as well, and intracellular digestion of them was evidently in progress, for nucleated red cells were found in different stages of disintegration.

In the splanchnopleure in contact with the yolk of this embryo, *granulocytes* were readily found in small groups, their characteristic acidophilic granules being stained with eosin. Most of these cells had indented nuclei and cytoplasm closely packed with granules, but in some instances similar granules appeared in the basophilic cytoplasm of mononuclear cells that were still recognizable as hemocytoblasts. This formation of granulocytes occurred in membranes of normal embryos of the same age and evidently was not caused by the presence of carbon. No granulocytes were found in the membrane adjacent to collections of injected carbon particles.

Changes in the Body of the Embryo

When carbon was injected into the tissue of an early embryo (no. 35 A, 3½ days old) *accelerated proliferation of cells* was evident at the site of the injury 3 hours after the injection. With superficial destruction of the ectoderm there was sometimes active proliferation of the adjacent cells of the mesoderm and a papillary mass formed by them projected from the wounded surface. These mesodermal cells had a rounded form and mitotic figures were numerous. When the injecting tip of the micropipette passed into the body cavity of an embryo of the same age (no. 36) and injured the mesothelium and underlying tissue adjacent to the bulbus arteriosus, proliferation of mesothelial and mesenchymal cells after 8 hours formed a small mass projecting into the body cavity. Carbon particles within this mass were found in mononuclear phagocytes.

When carbon was injected directly into the mesoderm, the embryonic connective tissue was spread apart by the injected material and erythrocytes were in places abundant, but even after 8 hours there was scant if any cellular reaction. Carbon particles were seen 3 hours after injection (no. 25), attached to the surfaces of the mesodermal cells and their processes and within their cytoplasm.

Mononuclear phagocytes containing carbon particles were found in embryos 3½ days old at the time of injury and fixed 8 hours (no. 35) and 24 hours (no. 36) later. They were found within masses of proliferating mesodermal cells. After 8 hours some of them had reached considerable size and contained vacuoles.

In embryos 3½ or 4¾ days old, examined 3, 8, 18 and 24 hours after injury caused by injection of India ink into the mesoderm of the chorion, no granulocytes were found. In embryos (nos. 40 and 41) 8 days old at the time of injection and examined 8 hours later, numerous *granulocytes* with characteristic eosinophilic granules and lobed nuclei were present about the injected carbon. Small round cells with a round nucleus and basophilic cytoplasm were present in the mesoderm adjacent to the injected carbon and in places had collected to form groups. In some of these cells, usually about small blood vessels, eosinophilic granules appeared in the basophilic cytoplasm (granuloblasts). As the granules increased in number, the cytoplasm lost its basophilic stain and various transitions between granuloblasts and granulocytes with bilobed or trilobed nuclei were found. The presence of carbon in the subcutaneous mesoderm had stimulated the new formation of granulocytes.

In one instance (no. 41, 8 days old and examined 8 hours after injection), India ink was introduced into the mesodermal tissues of the leg, and in the perichondrium on the side next to the injury there was an almost continuous row of granulocytes with eosinophilic granules. These cells occurred among the polygonal cells with basophilic cytoplasm that surrounded the cartilage. Granulocytes were abundant in the tissue between the site of injury and the perichondrium.

In older embryos (nos. 33 and 79), 12 and 17 days old at the time of injection, a few granulocytes were found about injected carbon after 3 hours. It is noteworthy that at this period of development granulocytes were readily found in the bone marrow and were present in small number in blood vessels but no evidence that they had migrated from blood vessels at the site of injury was obtained.

In an embryo (no. 94) 19 days old, an *inflammatory reaction similar to that of the adult* was in progress 10 hours after the injection of a considerable quantity of carbon. Some hemorrhage had occurred as the result of injury at the site of injection. Granulocytes in great number accumulated in and about clumps of carbon particles and were found in small number in the surrounding tissue. Here they were numerous within small veins and capillaries. They were often adherent to the endothelium, and granulocytes fixed in process of migration through the vessel wall were readily found. Granulocytes about the carbon had occasionally ingested a few particles. Mononuclear phagocytes were

present in small number, and these were laden with ingested carbon particles.

Inflammation Caused by Turpentine and Carbon Particles

When colorless material was injected into the body of an embryo it was often not possible to discover the site of injection after fixation, but when the material was mixed with carbon particles the site of injury was readily identified. Turpentine was diluted with an equal volume of olive oil and to the mixture was added approximately one-third of its volume of India ink. In two experiments (nos. 106 and 107) the mixture of turpentine, oil and India ink was allowed to dry and form a semisolid mass before it was applied to the site of a puncture into the embryo.

Changes in the Membranes

In one embryo (no. 106, 36 hours old when injected, and examined 11 hours later), carbon was found in and below the ectoderm of the amnion at its junction with the body of the embryo. Endothelial cells in the walls of small blood vessels in contact with clumps of carbon particles had undergone proliferation, which was limited to the part of the endothelial lining adjacent to the carbon. The endothelium was stimulated to form masses of cells projecting into the lumen of the vessel. These cells had round nuclei and basophilic cytoplasm and resembled hemocytoblasts.

In some instances (*e.g.*, no. 124, 3 days old when injected and examined 18 hours later) cells proliferating about clumps of carbon particles within the amnion have carried them to the interior of the cavity and thus brought about their elimination from the tissues of the embryo.

Changes in the Body of the Embryo

Below the surface of an embryo (no. 110) 48 hours old, examined 36 hours after injury, mesodermal tissue had undergone necrosis in a small focus in contact with the neural tube and here the overlying ectoderm and mesoderm had proliferated actively. *Accelerated proliferation* of ectoderm formed projecting papillae within which were keratinizing cells. Multiplication of mesodermal cells was accompanied by abundant new formation of intercellular fibers.

In an embryo (no. 123) 3 days old and killed 12 hours after injection, there was injury of mesodermal tissue below the dorsal surface of the embryo in contact with the neural tube, and here proliferation of fixed mesenchymal cells had occurred. Small round cells with basophilic cytoplasm and round vesicular nucleus infiltrated the injured tissue. These cells resembled hemocytoblasts on the one hand and the

macrophages present in the tissue on the other. These macrophages, which had a rounded outline, a single round nucleus and basophilic cytoplasm, contained nuclear fragments, refractive particles that stained with eosin and occasionally an ingested cell still intact.

In an embryo (no. 113) 12 days old and fixed 9 hours after injury, a few *mononuclear phagocytes* with characters similar to those just described contained carbon particles. In an embryo (no. 146) of the same age, but killed 24 hours after injection of the irritant, mononuclear phagocytes that had ingested carbon particles, red corpuscles and occasionally granulocytes were numerous and in some instances of large size. The origin of these phagocytes from cells with the characters of hemocytoblasts, which multiplied with mitosis and were situated just outside of small blood vessels, was readily traced. At first a small cell with round nucleus and basophilic cytoplasm had ingested a single erythrocyte. Cells with abundant ingested contents became larger, and often irregular in outline. As these cells increased in size, the basophilia of their cytoplasm was lost. It is noteworthy that similar transformation of hemocytoblasts into macrophages was seen within blood vessels of an embryo in which carbon had entered the blood stream and was widely distributed within the blood vessels.

Granulocytes have little part in the reaction that follows the injection of turpentine and carbon particles into early chick embryos. In embryos (nos. 106 and 107), 36 hours old when they received turpentine and carbon, and killed 11 hours later, there was active proliferation of mesodermal cells but no granulocytes were found. In an embryo (no. 123) 3 days old and killed 12 hours after injection, ectoderm and underlying mesoderm on the dorsal aspect of the embryo together with a small part of the neural tube were destroyed, but no granulocytes were found in or about the injured tissue.

In an embryo (no. 103) 6½ days old, and fixed 7½ hours after injection, there was no accumulation of granulocytes at the site of injury, but in an embryo (no. 112) of the same age, fixed 24 hours after injection, injury to the leg had destroyed ectoderm and underlying mesoderm, and there was hemorrhage in places. No carbon particles were found in the injured tissue. Granulocytes were fairly abundant but were limited to an area between the site of injury and the perichondrium of the rudimentary bone. Granulocytes, usually with lobed nuclei, were found here about small blood vessels. A few granulocytes were seen within the lumen of a small blood vessel and in several instances were fixed in the vessel wall itself, as though in process of migration. The number of granulocytes diminished as the actual site of injury was approached, and here none was found.

In an embryo (no. 146) 12 days old, fixed 24 hours after injection,

the site of injury was marked by carbon particles widely distributed and by hemorrhage of small extent. Granulocytes had accumulated and mononuclear phagocytes ingesting carbon particles were numerous. Within the wall and just outside of small blood vessels basophilic cells were in process of transformation into granulocytes, acidophilic granules being sparsely scattered in the basophilic cytoplasm of small cells with round nuclei (granuloblasts). The smallest cells that contained acidophilic granules had a diameter only slightly greater than the long diameter of an erythrocyte; the nucleus was round, and the cytoplasm conspicuously basophilic. No granulocytes were seen within the lumina of blood vessels adjacent to the injury, although granulocytes were abundant in well developed bone marrow in a bone of the leg.

In embryos 17 days old *inflammation similar to that in the adult* was produced by turpentine and carbon. Within 2 hours after injection of the irritant (no. 84), granulocytes collected about carbon particles and were migrating from blood vessels. In the adjacent tissue granulocytes with lobed nuclei were found in abundance within the lumina of small blood vessels. Others were fixed in transit through the wall. After 4 hours (no. 85) similar changes were seen, but after 6 hours (no. 80) accumulation of granulocytes about carbon particles was much more advanced. The granulocytes had taken up a few carbon particles, whereas macrophages adjacent to them were filled with carbon. Some local formation of granulocytes was in progress about small blood vessels, acidophilic granules being found in cells with round or indented vesicular nuclei and basophilic cytoplasm (granuloblasts). Where carbon particles had penetrated close to the periosteum of a leg bone, granulocytes with lobed nuclei were present in abundance in the periosteum and were found within the layer of osteoblasts next to the bone, but in the periosteum distant from the site of injury no granulocytes were found. In embryos (nos. 81 and 82) 17 days old and killed 10 hours after injury, granulocytes in the inflamed tissue were in great part mature with lobed nuclei, but some large granulocytes had a round nucleus and resembled myelocytes. In these embryos phagocytosis of granulocytes by macrophages was conspicuous and after 24 hours (no. 89) phagocytosis of granulocytes and of erythrocytes by macrophages was more advanced.

In an embryo 17 days old macrophages that had taken up carbon particles were found 6 hours after injection of turpentine and carbon (no. 80). After 10 hours (no. 81) mononuclear wandering cells with basophilic cytoplasm appeared in considerable number about blood vessels at the site of injury, and, as they became larger, vacuoles appeared in their cytoplasm. These cells took up carbon particles. Af-

ter 24 hours following the injection (no. 89), mononuclear phagocytes were very numerous and had ingested a large part of the carbon in the tissue. Cells that had taken up carbon contained some granulocytes and erythrocytes as well. Multiplication of mononuclear cells by mitosis had occurred about blood vessels, and from the blood vessel outward the transformation of these cells into macrophages was evident. They increased in size, lost their basophilic stain, became vacuolated, and ingested carbon particles.

Discussion

In embryos from 3 to 5 days old, traumatic injury accompanied by the introduction of particulate matter, namely, carbon, or of an inflammatory irritant such as turpentine causes accelerated proliferation of cells adjacent to the injury, both in the embryo itself and in its membranes. Similar changes are produced by carbon particles (India ink) alone and by turpentine with carbon, but they proceed more rapidly with the latter. Proliferation of ectodermal cells may cause the formation of papilla-like projections, with abnormal keratinization of cells. When ectoderm is destroyed, proliferation of mesodermal cells may produce small masses of tissue projecting above the surface. Carbon introduced by injection may be carried upward by proliferation of ectodermal or mesodermal cells below it and finally may be discharged upon the surface of the embryo. When an irritant is injected into the chorioallantoic cavity or into the body cavity, injury of mesothelium may be followed by proliferation of adjacent mesodermal cells to form a little mass projecting into the cavity. Carbon particles introduced into the tissue may be carried into the cavity by proliferation and desquamation of cells.

With carbon particles alone and with turpentine mixed with carbon particles, small masses of carbon have in some instances lodged just outside of a small blood vessel of the splanchnopleure next to the yolk and here the endothelium has been stimulated to form a small rounded mass projecting into the lumen. This little accumulation of proliferating cells is found next to the carbon particles but none is seen elsewhere.

Carbon particles introduced into the mesoderm of embryos 3 to 4 days old stick to the surface of mesodermal cells and their processes and they may enter the cytoplasm of cells. Save for accelerated proliferation of cells adjacent to the site of injury there is in these early embryos during several hours after injection of carbon particles little cellular reaction. Nevertheless, after 8 hours carbon particles are found ingested by round mononuclear cells that are vacuolated and resemble

histiocytes. The formation of macrophages has been observed in young embryos injected with turpentine and carbon particles. After 12 and 24 hours cells containing carbon particles are fairly numerous and like the macrophages of postembryonic life may contain simultaneously carbon particles, red blood corpuscles and occasionally granulocytes. They develop from small cells with round vesicular nuclei and basophilic cytoplasm not distinguishable from the hemocytoblasts that produce erythrocytes on the one hand or granulocytes on the other. The smallest of these cells are found about blood vessels and those that have ingested a single erythrocyte are readily identified by their basophilic cytoplasm. As they increase in size, the basophilic character of the cytoplasm disappears, vacuoles are abundant in them and the nucleus becomes oval or indented.

Formation of granulocytes from cells with a round vesicular nucleus and basophilic cytoplasm takes place normally in the chorionic membrane on the third day of development and mature granulocytes then enter the circulating blood (Sabin [15]). Formation of granulocytes in the bone marrow begins about the ninth day (Danchakoff [16]). In the rabbit, Sabin, Miller, Smithburn, Thomas and Hummel [17] have found that the number of leukocytes in the circulating blood is small throughout embryonic life, being about 900 before birth. Immediately after birth the number is approximately 2000, the increase affecting almost wholly the granulocytes.

A few round acidophilic granules make their appearance in the basophilic cytoplasm of extravascular cells which are not distinguishable from those intravascular cells that produce erythrocytes. These granuloblasts contain at first a few round granules which take a dull red stain with eosin and vary considerably in size. The granules later stain deeply, become elongated and fill the cell uniformly. The cytoplasm loses its affinity for basic dyes and the nucleus is round, oval, or indented and vesicular; these cells are myelocytes. More mature granulocytes have a horseshoe-shaped or lobed nucleus and are smaller than myelocytes. Both granuloblasts and myelocytes undergo division by mitosis.

In early embryos no granulocytes are found at the site of injury from 3 to 24 hours after injection of the irritant. The earliest embryo in which granulocytes have been found adjacent to the site of injury has been 6½ days old. Here none has been found at the actual site of injury in the leg, but they have been fairly numerous in a limited area between the injured tissue and the perichondrium surrounding the cartilage which at this period is the precursor of a bone of the leg.

In embryos 8 days old and in older embryos granulocytes have been found in moderate number about injected carbon after about 8 hours.

It is evident that they have been formed locally, presumably by stimulation of cells which can be transformed into them. Adjacent to the site of inflammation, granuloblasts in process of formation from cells with the characters of hemocytoblasts are found, singly and in groups, about small blood vessels. The smallest granuloblasts are round with a round vesicular nucleus and a variable number of clearly defined acidophilic granules within their basophilic cytoplasm. As acidophilic granules become more numerous the basophilia of the cell is lost and various transitions are found between cells with round or oval vesicular nuclei like those of myelocytes and granulocytes with characteristic polymorphous nuclei.

This new formation of granulocytes is limited to the site of inflammation and here cells with the morphological characters of hemocytoblasts found immediately about blood vessels are susceptible of transformation into granulocytes in response to the stimulus consequent upon the presence of an inflammatory irritant. It is noteworthy that cells with the same morphological characters may under other conditions be transformed into erythrocytes or into histiocytes, the former being formed within blood vessels and the latter, usually at least, extravascularly. Moreover, it is probable that under appropriate stimulus hemocytoblasts may produce granuloblasts within the lumina of blood vessels.

In certain tissues cells susceptible of transformation into granulocytes are more numerous than elsewhere. When inflammation has occurred in the neighborhood of the cartilage that is the precursor of long bones, granulocytes have been found in unusually large number in and about the perichondrium. It is possible that cells with the potentiality of forming the granuloblast of the bone marrow are numerous here and undergo prompt transformation into granulocytes under the stimulus of the inflammatory reaction.

In embryos from 17 to 19 days old, that is, shortly before hatching, inflammation acquires the characteristics of postembryonic life. Within 2 hours after injection of an irritant mature granulocytes have collected in considerable number about carbon in the tissue, are present within the lumina of small blood vessels and are fixed in the walls of vessels, presumably in passage through them. After 4 to 6 hours these leukocytes are more abundant and have ingested a few carbon particles, but at this time phagocytosis by macrophages is much more active. Nevertheless, formation of granulocytes in the tissue adjacent to the site of inflammation occurs as in younger embryos, for cells with a round nucleus, basophilic cytoplasm, and a few acidophilic granules are found about small blood vessels.

Summary and Conclusions

In embryos 3 to 5 days old, accelerated proliferation of cells is the most conspicuous reaction to injury by trauma or by the presence of irritants such as carbon particles or turpentine. Under the stimulus of these irritants papilla-like projections are formed by the ectoderm. With destruction of ectoderm, proliferation of mesodermal cells may form projections upon the surface, or with destruction of the mesothelium small masses of cells may project into the body cavity. Endothelium of a blood vessel may be stimulated to form masses of cells projecting into the lumen. Proliferation of cells below particulate matter that has entered the tissue may carry it to the external surface of the embryo or into the body cavity.

In early embryos, 3 days old, there is phagocytosis of particulate matter by mononuclear cells which have the characteristics of histiocytes and like them engulf and digest erythrocytes and other cells. In older embryos it is evident that these cells are in large part derived from perivascular cells with basophilic cytoplasm which have the structural characteristics of hemocytoblasts.

Granulocytes which are first formed in the somatopleure in contact with the yolk have little if any part in the reaction that follows the introduction of an inflammatory irritant into the tissues of early embryos and are first seen in small number at the site of inflammation in embryos from 6 to 8 days old.

Granulocytes that accumulate about an inflammatory irritant during embryonic life are in great part formed locally. The action of the irritant stimulates extravascular cells with the characteristics of hemocytoblasts to form acidophilic granules (granuloblasts). These cells, dividing by mitosis, produce at the site of inflammation myelocytes and mature polymorphonuclear granulocytes. At a very early period of development cells, of which the relation to the perichondrium suggests that they will take part in the formation of bone marrow, appear to be especially susceptible to transformation into granulocytes.

Cells morphologically resembling hemocytoblasts and widely distributed in the tissues of the embryo may be transformed by appropriate stimuli into histiocytes (macrophages).

Within a few days preceding hatching (17th to 19th day of embryonic development) inflammation assumes the character of postembryonic inflammation and granulocytes accumulate promptly and in large number by migration from blood vessels.

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INFLAMMATION IN EMBRYONIC LIFE

II. Infection of Chick Embryos With Avian Tubercle Bacilli *

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The avian tubercle bacillus is well suited to the study of inflammation in chick embryos because it is pathogenic for fowls and produces in them well known changes. In the following experiments the reaction produced by the microorganism has been studied both in the chorioallantoic membrane and in the tissues of the body of the embryo.

When Goodpasture and Anderson [1] inoculated the chorioallantoic membrane of the chick with avian tubercle bacilli, embryos that were 6 days old when inoculated died within 4 days, but older embryos lived until the time of hatching. After 24 hours polymorphonuclear leukocytes and a few mononuclear cells were found at the site of inoculation and after 48 hours mononuclear cells had increased in number. Both kinds of cells ingested tubercle bacilli, and some mononuclear phagocytes ingested leukocytes containing tubercle bacilli. In mononuclear cells tubercle bacilli were found in great number, and the authors believed that the microorganism multiplied within them. Tubercle bacilli were found in mesodermal cells that were far distant from larger accumulations of tubercle bacilli. Following inoculation, Costil and Bloch [2] found tubercle bacilli within epithelial cells of the ectoderm. Human tubercle bacilli produced, 7 days after inoculation, collections of cells with little resemblance to tubercles. Lesions produced by B. C. G. after 7 to 9 days showed some evidence of retrogression.

In embryos, inoculated with human tubercle bacilli when 12 days old and observed 6 days later, Moore [3] has described the formation of tubercles with caseation and giant cells. Atypical tubercles were produced by bovine tubercle bacilli. Avian tubercle bacilli invaded the mesoderm and were found in mononuclear phagocytes.

Methods

Eggs, after different periods of incubation, prepared by the procedure described in the preceding article,[4] have been inoculated with cultures of avian tubercle bacilli. Quantities of wet bacilli varying from 0.05 to 0.2 mg. have been used for inoculation. Sections stained with carbol fuchsin and counterstained with light green have been used to

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demonstrate tubercle bacilli. They have been compared with immediately adjacent sections cut in series and stained with hematoxylin and eosin-azure in order to demonstrate cellular structure in greater detail.

Inflammation Caused by Avian Tubercle Bacilli

Changes in the Membranes

When eggs, incubated for 6 days, were inoculated with avian tubercle bacilli and examined 6 (no. 159) or 8 (nos. 149 and 150) hours later, acid-fast bacilli were recognizable upon the surface of the chorioallantoic membrane in clumps usually surrounded by erythrocytes. A few were seen in ectodermal cells still attached to the membrane, others were in desquamated ectodermal cells and some were in round cells whose character was not definable. In embryos examined 8 hours after inoculation, tubercle bacilli were occasionally found in the mesoderm in or upon mesodermal cells. Twelve hours after infection (no. 151) some ectodermal cells, of which a few contained tubercle bacilli, had undergone proliferation and formed small projecting papillae. In the underlying mesoderm tubercle bacilli were seen in or upon mesodermal cells, and proliferation of these cells had occurred so that they were more numerous than elsewhere. On the surface of the ectoderm were round mononuclear cells containing tubercle bacilli, and these were in part, at least, histiocytes, for occasionally erythrocytes and tubercle bacilli were found within the same cell. Among the proliferating ectodermal and mesodermal cells of the chorion and among mononuclear cells found on the surface, granulocytes were moderately abundant, and several of them contained a few tubercle bacilli. Granulocytes on the surface had a bilobed or trilobed polymorphous nucleus, whereas in the underlying tissue granulocytes with a single vesicular nucleus were readily found and were most abundant about small blood vessels.

In an embryo 6 days old, 24 hours after infection (no. 152), ectoderm was lost in small areas and in others the cells had proliferated so that the margin about the defect was thickened. A few tubercle bacilli were found in the swollen ectodermal cells nearby. Below the site where the ectoderm was lost, mesodermal cells had multiplied in a circumscribed area, and here tubercle bacilli were found, but they were abundant only near the surface of the exposed mesoderm. Some were in cells that had anastomosing processes and were fibroblasts, but most of them formed compact clumps filling the cytoplasm of round cells of which the nucleus could be seen if tubercle bacilli were not too numerous. Mature granulocytes with lobed nuclei were found among the proliferating mesodermal cells and upon the surface of the mesoderm at

sites where ectoderm had been lost. An occasional granulocyte contained tubercle bacilli in small number.

In an embryo 6 days old, when examined 24 hours after infection (no. 169), tubercle bacilli had entered the chorioallantoic cavity and were found within the flat cells lining it. These cells had proliferated to form projecting mounds in which mesothelial and underlying mesoblastic cells were no longer distinguishable. The uppermost cells contained many tubercle bacilli, and cells, of which the cytoplasm was filled with bacilli, had become free in the overlying cavity. Tubercle bacilli that had penetrated downward into the mesoblast were found in contact with mesodermal cells and their anastomosing processes, and some bacilli were within the cytoplasm of these cells. Among the proliferating cells a few granulocytes were found. In a part of the membrane where it overlies the yolk, tubercle bacilli were abundant and here granulocytes were numerous. About blood vessels near the yolk, granulocytes were in process of formation. Mononuclear cells with basophilic cytoplasm contained a few round acidophilic granules (granuloblasts), and similar cells with numerous granules and no basophilic stain (myelocytes) were seen.

In an embryo 6 days old when infected, and killed 3 days later (no. 181), the only tubercle bacilli that were recognizable were within desquamated mesothelial cells free in the chorioallantoic cavity. Nevertheless, in places mesothelial and mesoblastic cells had undergone proliferation and were crowded together. Where this change had occurred, many mature granulocytes with lobed nuclei were found. They were seen within small blood vessels and were often adherent to the intima. In this part of the membrane there was no new formation of granulocytes, but in the membrane overlying the yolk sac hemocytoblasts filled the lumina of small blood vessels and similar cells were found outside of them. Acidophilic granules appeared in the basophilic cytoplasm of these cells and active new formation of granulocytes was evidently in progress.

In another embryo of the same age at the time of infection and examined 5 days later (no. 182), tubercle bacilli had multiplied actively and were found upon the surface of the ectoderm within desquamated cells, in ectodermal cells still attached and upon or within some of the underlying mesodermal cells. Where tubercle bacilli were abundant there was proliferation of both ectodermal and mesodermal cells. Granulocytes were very numerous and occasionally they contained tubercle bacilli. About small blood vessels granulocytes were in process of formation and cells with basophilic cytoplasm contained a few acidophilic granules which were round, larger and less brightly stained than

the elongated granules of mature avian granulocytes. Cells with the characters of myelocytes, as well as mature polymorphonuclear granulocytes, were found within the lumina of small vessels in the affected area.

In an embryo (no. 184), 11 days old at the time of injection and examined 48 hours later, there was active proliferation of ectodermal cells with formation of projecting mounds of cells and in places these cells had undergone keratinization. No tubercle bacilli were found except upon the surface, and here within granulocytes a few acid-fast bacilli were found. Granulocytes were abundant in the hyperplastic ectoderm and in the underlying mesoderm, and in the latter there was very active new formation of them. Granuloblasts were so numerous that 92 were counted about one small blood vessel cut tangentially, and here two of them were undergoing mitosis.

In one place within the mesoderm there was a lesion resembling an abscess, and here mature granulocytes with a few other cells occupied almost an entire field under low-power magnification. About this focus was a zone in which mononuclear cells predominated, although polymorphonuclear granulocytes were numerous. These mononuclear cells, in part with anastomosing processes, resembled embryonic fibroblasts and were closely crowded together. Less numerous were cells with basophilic cytoplasm resembling immature histiocytes. Granulocytes were in process of formation in the tissue surrounding the abscess, granuloblasts being found about small blood vessels and occasionally within their lumina. With the acid-fast stain, tubercle bacilli were not found within the area where granulocytes were most numerous nor in the surrounding zone of cell accumulation, but outside of the latter in apparently unaltered mesoblast a few tubercle bacilli were found in contact with mesodermal cells.

Changes in the Body of the Embryo

When avian tubercle bacilli were introduced into the body of an embryo (no. 168) 6 days old, they were found 6 hours later at the site of a defect in the ectoderm and in contact with the mesoderm. The only change that had occurred was some proliferation of ectodermal cells causing thickening of the ectoderm next to the defect. Tubercle bacilli that entered the mesoderm of embryos (nos. 163 and 164) 7 days old were found 6 hours after injection free in the mesoderm or in contact with mesodermal cells, but no reaction to their presence was evident.

In an embryo (no. 161) 6 days old when inoculated, and killed 24 hours after infection, tubercle bacilli were found in the mesoderm at a place where overlying ectoderm had been destroyed. Tubercle bacilli

were in contact with, and within, mesodermal cells with anastomosing processes. There had been some proliferation of these cells, as indicated by their increased number when compared with adjacent mesoderm and by the presence of mitotic figures. Here no macrophages and no granulocytes were found. In a circumscribed focus within the mesoderm close to the spinal cord, tubercle bacilli were present in such number that they were readily recognized with low-power magnification. Most of these tubercle bacilli were in round mononuclear cells and filled their cytoplasm. In the periphery of the focus tubercle bacilli were in contact with mesoblastic cells, but their number was small. Eosin-azure staining showed the presence of many round cells with basophilic cytoplasm, but granulocytes were not demonstrable.

In the same embryo tubercle bacilli had entered the blood stream, for within a small blood vessel adjacent to the focus just described a mononuclear cell contained several tubercle bacilli and in the liver were two foci in which cells had accumulated and in which tubercle bacilli were abundant. One of these foci was sharply defined because cells of mesoblastic type had replaced the columns of liver cells. Here round cells contained tubercle bacilli in great number, often filling the cytoplasm. Cells resembling fibroblasts contained the microorganism in smaller number, and fibers like those of reticulum were seen between the cells. In another place liver cell columns were intact and endothelial cells were apparently proliferating to form round cells that contained tubercle bacilli in large number. Restraint of the spread of tubercle bacilli was feeble; a few were found in flat endothelial cells lining small vessels, and some were present within liver cells.

In another embryo of the same age (no. 181), examined 48 hours after infection, there was evidence of dissemination by way of the blood. In this instance tubercle bacilli were found in an endothelial cell of the liver and several clumps of bacilli were within glomeruli of the kidney.

When tubercle bacilli had entered the body cavity of the embryo they were found in cells of the mesothelial lining. In one embryo (no. 152) 6 days old when infected and examined 24 hours later, mesothelial cells over the surface of the liver contained them, and within the cavity they were seen in mononuclear cells which were apparently in part desquamated lining cells. An occasional granulocyte was found in the abdominal cavity. In an embryo (no. 162) 48 hours after infection, necrosis of cells with nuclear fragmentation had occurred in the central part of a group of proliferating mesothelial cells in contact with the spleen. A few tubercle bacilli were found at the periphery of the necrotic area, but a larger number were in the immediately adjacent meso-

thelial cells, some of which were crowded with them. It is probable that some of the cells containing tubercle bacilli found within the body cavity were histiocytes, for in embryos (nos. 162 and 181) examined 48 hours after infection, mononuclear cells in this cavity contained erythrocytes as well as tubercle bacilli.

Embryos 11 days old at the time of infection and examined after 48 hours (no. 184) and after 81 hours (no. 185) have afforded opportunity to observe the local formation of granulocytes. In the former, tubercle bacilli were widely scattered in the mesoderm below a defect in the ectoderm of the leg. Here cells had accumulated in great number and in one small focus granulocytes were so numerous that the lesion resembled an abscess. Outside of this focus granulocytes were less numerous and mononuclear cells predominated. The latter were in part proliferating mesodermal cells and in part round mononuclear cells with basophilic cytoplasm. Tubercle bacilli were found in the abscess-like focus, in places filling the cytoplasm of mononuclear cells. At the periphery of the abscess where mononuclear cells predominated tubercle bacilli were not found, but in the relatively normal mesoblast outside of this area they were seen in or upon mesoblastic cells. In the zone in which mononuclear cells were abundant, new formation of granulocytes was proceeding actively, acidophilic granules being seen in mononuclear cells with basophilic cytoplasm (granuloblasts).

In embryos 18 days old at the time of inoculation and killed 24 hours later (nos. 214 and 216), bacilli had entered the subcutaneous tissue of the leg and the inflammatory reaction had the usual character of inflammation in birds and mammals. The tissue was edematous, and granulocytes had accumulated in considerable number. Round cells with a round or indented nucleus were present in smaller number, and some of them had ingested granulocytes. Both granulocytes and mononuclear cells had ingested tubercle bacilli, and occasionally it was evident that mononuclear cells contained granulocytes in which tubercle bacilli were recognizable. Granulocytes were seen within and about small vessels approximating capillaries in size and in places were engaged within the wall of the vessel. Migration of granulocytes was evidently in progress.

Discussion

Tubercle bacilli introduced into the amniotic cavity of embryos 6 or 7 days old invade ectodermal cells and may multiply within them. Hyperplasia of ectoderm may produce projecting papillae. On the contrary, the microorganism may cause necrosis of the ectoderm, and where ectoderm is destroyed, or perhaps without destruction of it, tu-

bercle bacilli may penetrate the mesoderm. Here they may be found in contact with fibroblasts or their anastomosing processes or within the cytoplasm of these cells. Their relation to the cells is like that observed with carbon particles.[4] At first there is no evident response to the presence of the organism, but within 12 hours mesoblastic cells proliferate. Bacilli are found within mesothelial cells lining the body cavity. The scant reaction that has occurred suggests that cells may have been invaded by the microorganism.

Within 12 hours after infection, tubercle bacilli are found in round cells with round or indented nuclei. The probability that these cells are histiocytes is increased by the observation that some of them contain both tubercle bacilli and erythrocytes or occasionally nuclear fragments.

In the chorioallantoic membrane of embryos 6 or 7 days old, granulocytes make their appearance within 12 hours after infection at sites where tubercle bacilli have caused proliferation or necrosis of ectoderm or of mesoderm, and a few of them may contain one or several tubercle bacilli. These cells have the polymorphous nuclei and elongated acidophilic granules of the mature granulocytes of fowls. In embryos of this age new formation of granulocytes is found during the early stages of the ensuing reaction only in that part of the membrane overlying the yolk where they are formed normally. In these early embryos that have lived 2 to 5 days after infection, granulocytes in process of formation have been found about small blood vessels at the site of injury. Evidently some of the granulocytes that accumulate here are formed locally. Granulocytes accumulate in the tissue adjacent to the injury; and granuloblasts, some in mitosis, are abundant, though none are found in corresponding parts of the membrane elsewhere. It is possible that a few granulocytes formed normally throughout the deeper part of the membrane reach the lesion by migration from blood vessels, but most of them are formed by cells that are stimulated by the irritant to form granuloblasts, and these in turn to form mature granulocytes. The cells from which these granuloblasts arise have a round nucleus and basophilic cytoplasm and resemble hemocytoblasts. Primitive cells, which form either erythrocytes, granuloblasts, or histiocytes, are indistinguishable under the condition of our study.

When avian tubercle bacilli have entered tissues of the body of embryos 6 or 7 days old they may cause proliferation of ectodermal or of mesodermal cells and in places necrosis of ectodermal cells may occur. After 24 hours a circumscribed lesion may be produced in the mesoderm. It is characterized by localized proliferation of embryonic fibroblasts and the appearance of many sharply defined round cells with round vesicular nuclei and basophilic cytoplasm. It is noteworthy that

most of the tubercle bacilli are contained in these round mononuclear wandering cells, whereas relatively few are found in or upon the fixed cells with anastomosing processes. Granulocytes accumulate less rapidly than in the membranes of the embryo and few, if any, are found after 24 hours in lesions produced by the microorganism. The lesion that is formed by proliferation of fibroblasts and accumulation of mononuclear phagocytes does not resemble a tubercle because it lacks the epithelioid cells that give the tubercle its characteristic form.

When tubercle bacilli have entered the body cavity they are found within the flat mesothelial cells that line it, and these cells undergo proliferation. It is probable that they are passively invaded, because tubercle bacilli have entered underlying liver cells as well. A focus of necrosis may be found within a mass of proliferating mesoblastic cells and is presumably produced by the action of the bacilli.

Tubercle bacilli in some instances have found their way into the blood stream and have been transported to the liver, where they are found in endothelial cells of blood vessels, and to the kidney, where they are lodged in glomeruli. In one experiment in an embryo 6 days old and examined 48 hours after inoculation, tubercle bacilli had been distributed by the blood stream, and lesions with some resemblance to tubercles were seen in the liver. In circumscribed foci, mononuclear cells contain tubercle bacilli in large number and are mingled with proliferating embryonic fibroblasts and newly formed collagen fibrils. Cells that are in contact with the endothelium of capillaries and are apparently analogous to Kupffer cells are proliferating and in part, at least, produce isolated mononuclear cells, of which the cytoplasm is filled with tubercle bacilli. The nodule that is formed has replaced the preexisting columns of liver cells, but it differs from a tubercle because epithelioid cells are not found. No granulocytes have been seen in these lesions.

Accumulation of granulocytes in lesions of embryos 11 days old has been found 2 and 3 days after infection. In one instance granulocytes have assembled in such great number that the lesion resembles an abscess and in the periphery of this focus there has been active localized new formation of granulocytes. Granuloblasts in considerable number are dividing by mitosis.

In early embryos, preceding the tardy cellular reaction that ensues, there is scant resistance to the multiplication of avian tubercle bacilli and to invasion by them for they are found in ectodermal cells, in or upon fixed cells of the mesoderm, within mesothelial cells and even within liver cells. With the appearance of mononuclear phagocytes, which are found long before granulocytes appear, tubercle bacilli are

often seen in such great number in their cytoplasm that multiplication of the microorganism within the macrophage is probable. After some time, mature granulocytes appear in the lesion and are derived in great part at least from granuloblasts formed locally in response to the presence of the infectious agent. Under some conditions that are not definable, granulocytes are so abundant that the lesion has the appearance of an abscess. Granulocytes ingest tubercle bacilli, but only a few are found within them. In lesions where granulocytes are numerous, few tubercle bacilli are found, and it is probable that their presence is indicative of a reaction capable of retarding invasion by the avian tubercle bacillus.

In embryos 18 days old, that is, shortly before hatching, inflammation caused by the avian tubercle bacillus, like that following introduction of carbon particles or turpentine into the tissues, is characterized by accelerated accumulation of granulocytes by way of the blood vessels and resembles that of postembryonic life.

Summary and Conclusions

Avian tubercle bacilli, introduced into the membranes or into the tissues of early chick embryos, invade both ectodermal and mesodermal cells and cause accelerated proliferation of them. Under some conditions necrosis may ensue.

In early embryos up to 6 days of age there is at first tardy reaction to the presence of the microorganism, but mononuclear wandering cells containing tubercle bacilli make their appearance after approximately 12 hours. The great number of tubercle bacilli within them is probably the result of intracellular multiplication.

In early embryos a few granulocytes formed in the chorion in contact with the yolk sac may reach the site of inflammation by way of the blood stream, but most of those that accumulate about the microorganism in embryonic membranes or later in tissues of the body of the embryo are formed locally. Cells with the morphological character of hemocytoblasts are directly stimulated by the infectious agent to form granuloblasts characterized by basophilic cytoplasm containing a few acidophilic granules. These granuloblasts multiply by mitosis and in turn produce locally both myelocytes and mature granulocytes.

Circumscribed nodules are produced in the tissues of the embryo by proliferation of embryonic fibroblasts and accumulation of mononuclear wandering cells which ingest tubercle bacilli, but the lesion does not have the characteristics of a tubercle because epithelioid cells are not formed.

Granulocytes mobilize in increasing number during the last few days of embryonic life by migration from blood vessels and inflammation assumes its postembryonic character. Resistance to multiplication and invasion of avian tubercle bacilli increases with local increase in the number of granulocytes.

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ACQUIRED BICUSPID AORTIC VALVES WITH RETRACTED HORIZONTAL RAPHES *

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Although in most bicuspid aortic valves the raphe which divides the conjoined cusp is retracted into the sinus of Valsalva, the commissural attachment is not appreciably lowered. Nevertheless, certain examples occur in which the entire raphe is situated deep in the sinus of Valsalva and has a horizontal upper border attached to the aortic wall at a point distinctly below the normal position of the original commissure. This constitutes a distinct type of acquired bicuspid aortic valve.

REPORTS OF CASES

Case 1

A white male, 59 years old, was admitted to the hospital on March 4, 1940, and died on March 9, 1940. He complained of severe epigastric pain radiating to the back and shortness of breath of 5 days' duration. The heart was enlarged and the rhythm irregular but there were no murmurs. The blood pressure was 125 systolic and 60 diastolic. No history of rheumatic fever was obtained. The clinical diagnosis was either mesenteric thrombosis or dissecting aneurysm of the aorta.

The main pathologic diagnoses were idiopathic medial necrosis of the aorta with dissecting aneurysm of the thoracic and abdominal portions, left hemothorax (2.8 L.), cardiac hypertrophy and dilatation (700 gm.), syphilitic aortitis, and rheumatic heart disease with chronic endocarditis of the left atrium, mitral and aortic valves and formation of an acquired bicuspid aortic valve.

The bicuspid aortic valve consisted of one large cusp, 5 cm. in length, formed by complete fusion of the right and noncoronary cusps, and a smaller left cusp, 2 cm. long (Fig. 1). The triangular space between the two fused cusps on their ventricular aspect was virtually obliterated. The conjoined cusp was evenly subdivided at commissure B † by a narrow fibrous raphe measuring 5 by 2 by 1 to 2 mm.‡ This was markedly retracted and had practically a horizontal position at the

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† The following nomenclature of the aortic valve is used: The aortic cusps are designated according to the situation of the coronary arteries as the left, the right and the noncoronary cusps. The left-right commissure is referred to as commissure A, the right-noncoronary commissure as commissure B, and the left-noncoronary commissure as commissure C.

‡ These measurements indicate respectively the length (from origin to insertion), the width and the height (from the floor of the sinus of Valsalva to the superior surface) of the raphe.

bottom of the sinus of Valsalva. Proximally it originated from the aorta only 2 mm. above the attachment of the aortic valve and, after a linear course, it inserted into the base of the conjoined cusp. The outer surface was rounded, symmetric, approximately of uniform width and revealed no fissure. Just above the raphe there was a barely perceptible and irregular longitudinal elevation of the aorta.

Both aortic cusps were slightly thickened, especially the outer part of the conjoined cusp near the raphe. There was no calcific deposit. The free edge of the conjoined cusp presented a concave aspect toward the aorta. Commissures A and C showed no change. The right coronary ostium was situated 4 mm. above the commissural level while the left was in the usual position.

A longitudinal microscopic section through the middle of the raphe showed dense connective tissue.* Slight vascularity with capillaries and arterioles was present in the basal portion of the distal segment, just above the subaortic angle. The annulus fibrosus at the proximal extremity of the raphe was entirely anterior to the terminal elastic wedge of the aorta.†

The aortic cusps were the seat of diffuse fibrosis and also showed reduplication of the elastica in the ventricularis layer. However, no vascularity, exudate, or calcific change was present.

There was nodular thickening of the mitral valve along the line of closure. Microscopically the leaflets showed fibrosis and thick-walled blood vessels in the free portion. Although the left atrium was grossly negative, sections revealed elastic reduplications of the endocardium. The tricuspid and pulmonary valves showed no significant gross or microscopic change. The pericardium and myocardium were not remarkable.

Case 2

A white male, 64 years old, was admitted to the hospital on January 10, 1941, and died on January 15, 1941. He complained of severe substernal pain and shortness of breath of 1 week's duration. Examination of the heart was unsatisfactory because of marked pulmonary edema. The blood pressure was 100 systolic and 90 diastolic. No history of rheumatic fever was obtained. The clinical diagnoses were coronary thrombosis, myocardial infarction and acute pulmonary edema.

The main pathologic diagnoses were marked coronary arteriosclerosis with complete thrombotic occlusion of the left descending and right circumflex vessels, remote and recent myocardial infarction, cardiac

* Microscopic sections were studied after hematoxylin and eosin staining, the Weigert technic for elastic tissue and the combined Weigert and van Gieson methods for elastic and connective tissue.

† When the annulus is anterior to (or in front of) the aortic wedge, it is separated by the latter from the pericardium. If the annulus be posterior to (or behind) the wedge, it is in contact with the pericardium.

hypertrophy and dilatation (425 gm.), and chronic rheumatic heart disease with mitral, tricuspid, pulmonary and aortic valvulitis, formation of an acquired bicuspid aortic valve and calcific disease of the aortic valve with stenosis.

The aortic valve consisted of two cusps of equal size, each measuring 3.5 cm. in length (Fig. 2). One was a combined right and noncoronary cusp which presented an uninterrupted concave aspect toward the aorta. Commissure B was markedly retracted in the sinus of Valsalva and at its site was a centrally located, almost horizontally disposed raphe, calcified throughout its length. The raphe measured 8 by 2 to 3 by 3 mm., arose proximally from the aorta 3 mm. above the attachment of the valve and inserted distally into the base of the conjoined cusp. Its surface was nodular and irregular owing to calcific deposit and showed no fissure. There was no lesion of the aortic wall of the sinus of Valsalva above the raphe.

Both aortic cusps were rigid and showed calcific deposit. The sinuses of Valsalva were partly filled with calcified nodules. At commissure A there was slight fusion between the left and right cusps. Commissure C showed no change. The coronary ostia occupied their usual positions.

A transverse section of the raphe 3 mm. from its proximal end showed numerous calcific nodules but no vascularity or exudate. A longitudinal section of the entire raphe revealed diffuse calcific deposit. Vascularity and exudate were found principally in the ventricularis layer and especially distally in the region above the subaortic angle. Proximally the aortic media terminated entirely behind the fibrous tissue of the annulus. The calcific nodules in the aortic cusps were accompanied by focal vascularity with capillaries and exudation of lymphocytes.

The mitral, tricuspid and pulmonary valves, the left atrial endocardium, and the pericardium showed no significant gross change. Of numerous sections of the mitral valve, a few revealed vascularity and fibrosis of the ring and proximal free portion. The pulmonary valve was similar, while the tricuspid showed diffuse vascularity extending almost to the line of closure. There were no rheumatic lesions in the left atrium or myocardium.

Case 3

No clinical history was available.

The main pathologic diagnoses were marked coronary arteriosclerosis with complete thrombotic occlusion of the right circumflex artery, remote posterior basal infarct of the left ventricle, and chronic rheumatic heart disease with mitral and aortic valvulitis, formation of an acquired bicuspid aortic valve and calcific disease of the aortic valve.

The bicuspid aortic valve consisted of one cusp 4 cm. in length, formed by complete fusion of the right and noncoronary cusps and a smaller left cusp 3 cm. long (Fig. 3). The triangular space between the two fused cusps was obliterated. The conjoined cusp presented an uninterrupted concave aspect toward the aorta. It was evenly subdivided by a horizontal calcified ridge measuring 15 by 2 to 7 by 5 mm. This was situated deep in the sinus of Valsalva, its proximal end being attached to the aorta 8 mm. below the commissural level and its distal extremity inserting into the conjoined cusp midway between the ring and free edge. There was lateral bulging of the middle of the raphe resulting in a peculiar ovoid form. The outer surface was slightly nodular and revealed no fissure. The aorta above the proximal end showed no significant change.

Both aortic cusps were slightly thickened. There was fusion of the left and right cusps with calcific deposit in the raphe. Nodules of calcification were also present in the sinus of Valsalva. Commissure C showed no change. The coronary ostia occupied their usual positions.

Transverse sections of the raphe, 3 mm. from the proximal and the distal ends respectively, showed a similar picture. The calcific deposit was marked. There were vascularity and exudate in the lateral basal regions, *i.e.*, the attachments of the cusps, and in the distal raphe the vessels extended into the outer portion of the ventricularis layer.

A longitudinal section of the middle of the raphe revealed dense connective tissue containing numerous calcific nodules. Most of the calcium was deposited on the aortic side in the fibrosa layer. Vascularity was most prominent in the distal portion along the ventricularis layer, especially in and near the attachment of the valve. There were several thick-walled arteries. The aortic media was behind the annulus fibrosus at the commissural attachment.

The aortic cusps showed calcific nodules in all layers, accompanied by an infiltration of lymphocytes, and vascularity with capillaries and arterioles. There were prominent subaortic elastic reduplications.

The mitral valve was grossly negative except for a ridge of calcium extending from the noncoronary aortic cusp to the ring and base of the anterior leaflet. Microscopic sections showed focal calcific deposit, lymphocytic exudate and vascularity of the proximal free portion of both leaflets. The tricuspid and pulmonary valves, the left atrium, the pericardium and myocardium revealed no gross or microscopic evidence of rheumatic fever.

Case 4

A white man, 63 years old, was admitted to the hospital on March 28, 1941, and died on April 15, 1941. He gave a history of progressive shortness of breath for the

past 4 years and ankle edema for 1 year. Twenty years ago he was told by a physician that he had heart trouble. There was no history of rheumatic fever. The heart was enlarged, the rhythm regular and the blood pressure 110 systolic and 80 diastolic. Over the aortic area was a rough systolic murmur transmitted upward, and a faint aortic second sound but no diastolic murmur. The roentgenogram showed calcification in the region of the aortic valve. The clinical diagnoses were rheumatic heart disease with calcific aortic stenosis, auricular fibrillation and congestive heart failure.

The principal diagnoses at autopsy were chronic rheumatic heart disease with aortic valvulitis and formation of an acquired bicuspid aortic valve, calcific disease of the aortic valve with stenosis, and cardiac hypertrophy and dilatation (630 gm.).

The aortic valve consisted of two cusps of equal size, each measuring 3 cm. in length (Fig. 4). One was a combined right and noncoronary cusp, evenly subdivided by a raphe at commissure B. The raphe consisted of a calcified bar measuring 14 by 3 by 3 to 4 mm. and formed a horizontal ridge deep in the sinus of Valsalva. Its proximal attachment to the aorta was situated 1.2 cm. below the commissural level and after a linear course it inserted into the lower third of the conjoined cusp. The outer surface was slightly nodular and showed no fissure. There was no suggestion of a ridge in the aorta above the proximal end.

Both aortic cusps were rigid and practically immobile due to extensive calcific deposit. The sinuses of Valsalva were partly filled with calcific nodules. Commissures A and B showed no change. The coronary ostia were in the usual position.

Transverse microscopic section of the proximal raphe revealed calcific change but no vascularity or exudate. A transverse section of the distal raphe was similar except that the lateral basal regions contained capillaries and arterioles. Longitudinal microscopic section of the middle of the raphe revealed diffuse deposit of calcium in all layers, especially on the aortic side. Capillaries and an occasional arteriole were found along the base in the ventricularis layer. The terminal media of the aorta was distorted by the calcific process and calcified nodules even extended up behind it for a short distance. Nevertheless the elastic wedge remained posterior to the annulus fibrosus.

The aortic cusps revealed fibrosis, calcific deposit, diffuse vascularity and infiltration of lymphocytes. There were well developed elastic reduplications, some of multiple type, in the subaortic angle.

The mitral valve was grossly normal except for extension of a few calcific nodules from the aortic valve to the anterior leaflet. Microscopically there was no evidence of rheumatic fever. The tricuspid and pulmonary valves, the left atrium and the pericardium showed no significant gross or microscopic change.

Case 5

A white male, 64 years old, was admitted to the hospital on December 5, 1941, and died suddenly on December 12, 1941. He complained of progressive shortness of breath and edema of 1 year's duration. For the past 10 years he had had moderate dyspnea on exertion. There was no history of rheumatic fever. The heart was enlarged, the rate 40, the rhythm regular and the blood pressure 132 systolic and 98 diastolic. Over the aortic area was a harsh systolic murmur transmitted to the neck and absent aortic second sound, but no thrill or diastolic murmur. An electrocardiogram showed complete heart block. The clinical diagnoses were arteriosclerotic heart disease, aortic stenosis, cardiac enlargement, heart block and cardiac failure.

The main pathologic diagnoses were rheumatic heart disease with chronic mitral, pulmonary and aortic valvulitis with formation of an acquired bicuspid aortic valve, calcific stenosis of the aortic valve with extension of calcification into the membranous interventricular septum, and cardiac hypertrophy and dilatation (525 gm.).

The aortic valve consisted of two cusps of equal size, each measuring 3.8 cm. in length (Fig. 5). One was a conjoined cusp formed by fusion of the left and noncoronary cusps and it presented a continuous concave aspect toward the aorta. Deep in the sinus of Valsalva was a calcific horizontal ridge situated 1.2 cm. from commissure A and 2.6 cm. from commissure B. It measured 10 by 3 to 5 by 4 mm., arose proximally from the aorta just above the attachment of the aortic valve and inserted distally into the base of the conjoined cusp. There was no lesion of the aorta above the proximal end. The distal portion of the raphe was wider than the proximal. The outer surface was nodular because of calcific deposit and showed no fissure.

Both aortic cusps revealed diffuse calcific disease and were rigid and practically immobile. Calcific nodules filled the sinuses of Valsalva and also projected from the cusps at the line of closure. There was fusion of the left and right cusps for a distance of 5 mm. Commissure B showed no change. The left coronary ostium was situated 5 mm. above the commissural level while the right ostium was in its usual position.

Longitudinal microscopic section of the raphe of the conjoined cusp showed calcific deposit throughout its length. There was vascularity in the vicinity of the calcium and also along the base of the raphe in the ventricularis layer. Several thick-walled arteries were present. There was a prominent focus of vascularity distally near the subaortic angle. The aortic cusps showed diffuse fibrosis, calcification, foci of cartilage and osteoid tissue, exudate of lymphocytes and vascularity with capillaries and arterioles. There were occasional thick-walled arteries.

The mitral valve showed thickening at the line of closure and focal calcific deposit in the posterior leaflet. Microscopic sections revealed

chronic mitral and pulmonary valvulitis. There were no other stigmas of rheumatic fever.

Summary of Cases

The patients were all white males and their ages ranged from 59 to 64 years. In 2 cases there was a clinical diagnosis of aortic stenosis. None of the patients gave a history of rheumatic fever.

Gross Appearance of the Cusps

In 4 cases the conjoined cusp was formed by fusion of the right and noncoronary cusps and in 1 by fusion of the left and noncoronary cusps. In 3 cases the conjoined cusp was equal in length to the remaining cusp and in 2 it was larger. The conjoined cusp was evenly subdivided by the commissural raphe in 4 instances; in 1 case it was subdivided unequally into a small noncoronary and a larger left coronary segment. In all instances there was marked or almost complete obliteration of the triangular space below the raphe, and the concave aspect of the free edge of the conjoined cusp toward the aorta was continuous. The other commissures of the aortic valve showed fusion in 3 cases, *i.e.*, at commissure A in each instance.

The aortic cusps showed pathologic change in all cases. In 3 instances there was marked calcific disease and aortic stenosis. Calcific change without stenosis was present in 1 case and thickening without calcific deposit in 1 case.

The Commissural Raphe

Grossly the raphe consisted of a firm ridge of tissue situated deep in the sinus of Valsalva and occupying a horizontal or nearly horizontal position in the sinus (perpendicular to the long axis of the aorta). The raphe occurred at commissure B in 4 cases and at commissure C in 1 case. The proximal attachment to the aorta was generally only a few millimeters above the attachment of the aortic valve and the distal insertion was into the base or midportion of the conjoined cusp.

In 4 of the 5 cases the raphe was calcified throughout its length. It was usually either of uniform width or wider distally than proximally; in 3 cases the shape was irregular owing to calcific deposit and the surface was nodular. None of the lesions showed a fissure in the surface. The aorta above the raphe was smooth in 4 cases, while in 1 case it showed a barely visible, longitudinal elevation. The latter was readily distinguished from the congenital ridge described below.

Microscopically the raphe revealed dense connective tissue and usually calcific nodules. There was no significant elastica. In the longi-

tudinal sections vascularity and sometimes exudate were present along the base in the ventricularis layer, especially distally, and were generally prominent in the region of the attachment of the valve. Transverse sections revealed vessels in the lateral basal region and especially in the distal segment. In all cases the aortic media terminated behind the annulus fibrosus at the proximal end of the raphe. In 2 instances there was irregularity of the wedge owing to calcific deposit and inflammation.

Lesions of Rheumatic Fever

Rheumatic stigmas were present in all aortic valves. Particular attention was paid to stigmas of the mitral valve and multiple microscopic sections of both the anterior and posterior leaflets were made. Grossly the valve was normal in 3 cases and showed thickening without commissural fusion in 2 cases. Microscopically, however, 2 of the 3 grossly normal valves showed inflammation in the form of vascularity of the free portion, fibrosis and exudate. In the series, 4 of the 5 cases revealed conclusive microscopic evidence of chronic mitral valvulitis, probably rheumatic. In 3 cases rheumatic lesions were observed in the heart elsewhere than in the mitral and aortic valves; *i.e.*, in the left atrium in 1 case, in the tricuspid and pulmonary valves in 1 case and in the pulmonary valve alone in 1 case.

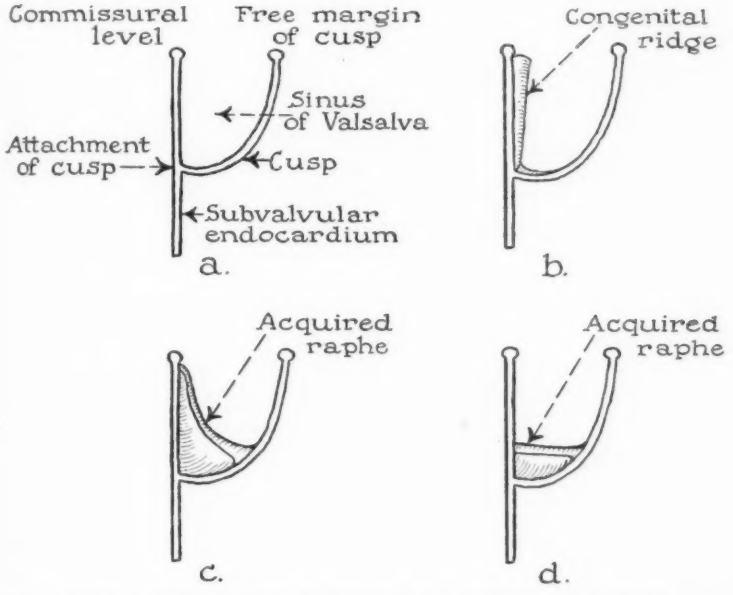
Comment

The bicuspid aortic valve described in this paper is produced by inflammatory fusion of two aortic cusps. Following adhesion certain changes occur: (1) the triangular space between the cusps is gradually obliterated; (2) the free margin of the conjoined cusp develops an uninterrupted concave aspect toward the aorta; (3) the commissural raphe is retracted into the sinus of Valsalva. Grossly the conjoined cusp then appears to be a single cusp.

The present lesion differs from the common variety of acquired bicuspid valve only in respect to the peculiar location and direction of the commissural raphe (Text-Fig. 1, d). This is uniformly retracted along its entire length so that it occupies a horizontal or nearly horizontal position at the bottom of the sinus of Valsalva. When retraction is especially marked, the raphe forms merely a small and inconspicuous ridge overlying the attachment of the aortic valve. Proximally it is attached to the aorta deep in the sinus and after a linear course inserts distally into the base of the conjoined cusp. The outer surface is smooth and rounded, or nodular and distorted owing to calcific deposit. Although not present in these cases, raphes of triangular shape suggesting

the outline of the fused cusps, and those showing a fissure with or without preservation of the cusp margins, probably occur.

The horizontal position of the raphe is evidently due to downward displacement of the proximal extremity from its original location at the commissural level. That the proximal origin of the raphe is actually situated at a commissure is indicated by the fact that the relationship between aortic media and annulus fibrosus is the same as occurs at a

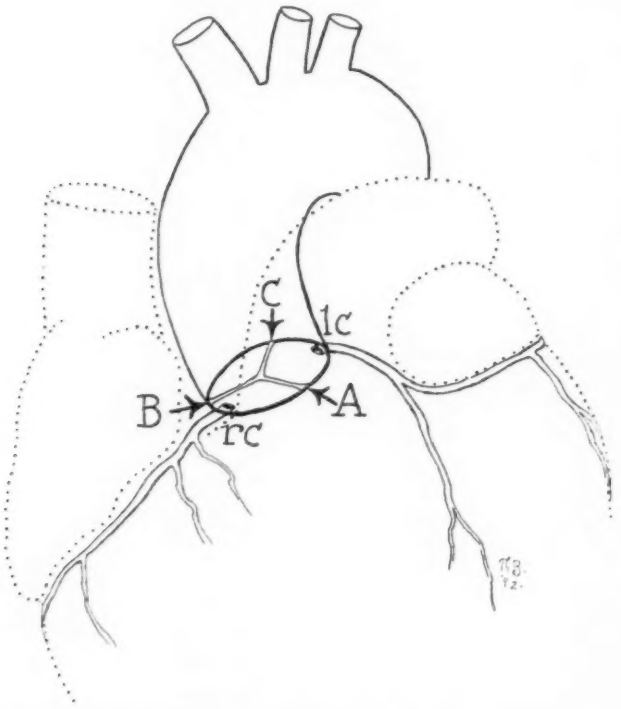


Text-Figure 1. Drawings of longitudinal sections of aortic valve to show the position in the sinus of Valsalva of the congenital ridge and the commissural raphes of acquired bicuspid aortic valves: a = normal valve; b = congenital ridge; c = acquired commissural raphe, usual type; d = retracted, horizontal type of acquired raphe.

commissure. As far as is known, location of a commissure at the bottom of the sinus of Valsalva does not occur normally.

The aorta, at about the point of attachment of the raphe, rather than the raphe itself, appears to be displaced downward. This displacement is presumably due to stretching of the aorta in this region, but the exact mechanism of the change of position is not clear. The fact that the lesion occurs most often at commissure B may be significant. In chronic rheumatic aortic valvulitis without bicuspid deformity, slight depression of commissure B is more frequent than at commissures A and C. There are somewhat hypothetic explanations, both physiologic and anatomic. The location of commissure B, to the right and in the con-

cave aspect of the longitudinal curve of the aorta and at a lower horizontal level than commissures A and C, is such that pressure exerted during diastole is possibly greater than at the other commissures (Text-Fig. 2); this effect might well be increased by the presence of disease in this region. It is also possible that the structure at commissure B is such that support against diastolic pressure is less than at commissure A or C.



Text-Figure 2. Drawing of aortic valve to show the position of the commissures *in situ*. The valve is inclined upward and to the left. Commissure B is situated to the right and in the concave aspect of the longitudinal curve of the ascending aorta, and at a lower horizontal level than commissures A and C.

In the usual type of acquired bicuspid aortic valve, the raphe is situated most often at commissure A, infrequently at commissure B and rarely at commissure C. The proximal extremity remains attached to the aorta at the commissural level, while the distal portion is retracted and inserts into the base of the conjoined cusp. Hence the direction in the sinus of Valsalva is generally oblique (Text-Fig. 1, c). Usually this raphe is symmetric, of greater width distally than proximally, and pre-

sents a rounded or smooth outer surface. However, the appearance may vary considerably, especially when there is superimposed calcific deposit, so that the raphe is irregular, nodular, or even distorted in shape.

Bicuspid lesions with low horizontal raphe are apparently much less frequent than those with oblique raphe, occurring in the ratio of about 1 to 5. A few additional cases have been encountered which appear to demonstrate an intermediate stage between those with no downward displacement of the commissural attachment and those described in this paper. In these intermediate cases the point of commissural attachment is slightly but not markedly below the upper border of the sinus of Valsalva.

Microscopically the commissural raphes of acquired bicuspid aortic valves are similar in appearance regardless of type.[1] They are composed of dense hyalinized connective tissue, may or may not show calcific deposit and have little or no elastica. In longitudinal sections vascularity and sometimes exudate are present in the ventricularis layer along the base of the raphe, especially in the distal segment, and are usually most prominent in the region of the attachment of the valve and the subaortic angle. In transverse section, especially of the distal portion, vessels may be seen in the lateral or basal region, corresponding to the attachment of the fused cusps.

Of importance is the relation of the annulus fibrosus to the terminal aortic wedge behind the proximal raphe. Although the raphes generally occur at the center of a conjoined cusp, they represent commissural lesions and hence should preserve the usual relation at the commissure, *i.e.*, termination of the aortic medial wedge posterior to the annulus. This was found to be true in all of the present cases, even though in two instances the terminal elastica was irregular and distorted by calcific deposit and inflammation. With very marked distortion, however, it may not be possible to determine this relation with certainty.

To be distinguished from acquired bicuspid aortic valves are those of congenital origin. The latter are uncommon in adults, and may be subdivided into those consisting of two normal cusps and those in which one of the cusps is divided by a ridge of congenital origin into two segments. The congenital ridge consists of a long, narrow, barlike elevation of the aorta, which projects only slightly into the sinus of Valsalva, is directed in the long axis of the aorta and is of uniform width and depth (Text-Fig. 1, b). Microscopically, it consists almost entirely of elastica whorled centrally and continuous laterally with that of the aortic media.[2-4]

A distinction between congenital and acquired bicuspid aortic valves

is not possible on the basis of the conjoined cusps themselves. In general appearance, size and location these may be entirely similar in the two lesions. The distinction depends on the differences in structure, both grossly and microscopically, between the congenital ridge and the acquired commissural raphe. These have already been indicated. Also of aid in differentiation is the aortic media-annulus fibrosus relation. In the acquired raphe the media lies behind the annulus, thus preserving the normal commissural relation. In the congenital ridge the annulus is generally overlapped both in front and behind by the terminal aortic wedge, with the posterior overlap lower than the anterior. Occasionally the annulus lies either entirely in front of the wedge or entirely behind it.

When calcific disease is present, the acquired lesion may be difficult to distinguish from the simple congenital bicuspid valve. This is especially true when the raphe is inconspicuous, situated very deeply in the sinus of Valsalva and largely obscured by calcific nodules in the sinus; the lesion might then appear to be of simple congenital type with calcific change. In most instances, however, the raphe can be identified grossly and established microscopically as an acquired commissural lesion by the aortic media-annulus fibrosus relation. If the raphe be completely obscured by the calcific deposit, a distinction between the two lesions may be impossible.

Occasionally, confluent nodules of calcification in the sinus of Valsalva form a ridge which simulates the commissural raphe. However, this pseudoraphe is grossly irregular and poorly defined in contrast to the sharply outlined true raphe. Moreover, should the ridge be situated in the central portion of the cusp, which is the usual position of the commissural raphe, microscopic section would reveal a terminal aortic media in front of the annulus rather than behind it. If the location is lateral to or near the commissure, microscopic study might not be of differential aid, since here the aortic media may lie behind the annulus normally.

The acquired bicuspid aortic valve is the result of an inflammatory process which is in all probability rheumatic in origin. Gross [5] has reviewed the evidence for a rheumatic etiology. This rests on the structure of the commissural raphe and also on the presence of stigmas of rheumatic fever, both in the aortic valve and elsewhere in the heart.

Horizontal raphes, similar grossly and microscopically to the present lesion, occur in rheumatic aortic valves which show commissural fusion but not bicuspid deformity. Here, also, the horizontal position of the raphe is probably due to lowering of its commissural attachment. Such

valves resemble the bicuspid lesion, but differ from it in two respects: (1) the triangular space below the raphe is only partly obliterated, and (2) the concave aspect of the fused cusps is interrupted opposite the raphe where it is convex.

The pathologic changes in the bicuspid aortic valves, namely, diffuse thickening and calcific deposit, are morphologically indistinguishable from those which occur in rheumatic fever. This includes such items as fibrosis involving all layers of the cusps, location of the calcific nodules principally in the fibrosa layer, vascularity and exudate in the ventricularis layer, and the presence of thick-walled blood vessels with muscular coats in the attachment and free portion of the cusps. Most recent studies support the view that calcific disease of the aortic valve has an inflammatory basis and is not degenerative in origin.[6-8]

In hearts with chronic or healed rheumatic fever, the stigmas of the disease are usually widespread. There are characteristic gross and microscopic changes. The gross lesions include various degrees of thickening, shortening and commissural fusion of the valves, especially the mitral and tricuspid; thickening and adhesion of the chordae tendineae; nodular thickening and wrinkling of the left atrial endocardium above the posterior mitral leaflet, and fibrous pericardial adhesions, especially in the atrioventricular sulci. Characteristic microscopic lesions consist of vascularity, exudate and fibrosis in the ring and free portion of the valves, involving especially the auricularis layer of the mitral and tricuspid valves and the ventricularis layer of the semilunar valves; vascularity and reduplication of the elastica of the endocardium of the left atrium and the subaortic angle, and Aschoff nodules in the myocardium.

In the present study, only unequivocal lesions were accepted as rheumatic stigmas. Attention was directed particularly to inflammation of the valves, especially the mitral and tricuspid. In situations other than the aortic valve, gross manifestations of rheumatic fever were present in only 2 cases and consisted of chronic or healed nondeforming mitral valvulitis. Microscopically, there were conclusive rheumatic lesions outside the aortic valve in 4 cases. The fifth case presumably represents an instance of rheumatic heart disease with residual lesions demonstrable only in the aortic valve.

The clinical significance of the condition is that of acquired bicuspid aortic valves in general. The bicuspid lesion usually occurs in hearts which are the seat of only mild or slight rheumatic disease.[1] In most cases, for example, the accompanying disease of the mitral valve is of the nondeforming type rather than productive of mitral stenosis. Furthermore, the bicuspid aortic valve is only rarely encountered in chil-

dren or young adults dead of florid rheumatic fever or in adults who have chronic rheumatic heart disease with marked deformity of two or more valves and cardiac failure.

The bicuspid valve results from fusion of two adjacent aortic cusps to form a single conjoined cusp. This pathologic change probably takes place within a relatively short period of time during childhood or early adult life. It is probable that functional alteration occurs, *i.e.*, stenosis due to adhesion, or insufficiency due to retraction of cusps, but is transient in nature and disappears when the bicuspid lesion is complete. The single and fused cusps evidently undergo stretching and as a result become functionally competent in systole and diastole. Thus the bicuspid lesion *per se* has no permanent effect on the heart.

Individuals with acquired bicuspid aortic valves are, however, prone to develop calcific disease of the valve. This development is an integral part of the underlying rheumatic valvulitis. The calcific deposit leads to rigidity of the cusps and in severe cases to stenosis of the aortic valve and eventual cardiac decompensation. Since the deposition of lime salts occurs slowly over a considerable period of time, functional valve disease is generally not apparent until later life. This was true of the cases in the present study.

Another disease, which may be superimposed on the acquired bicuspid aortic valve, is bacterial endocarditis, either acute or subacute. Presumably this is due largely to the rheumatic origin of the bicuspid lesion. Although none of the acquired lesions in this study showed bacterial disease, the latter is not infrequent in my experience in the usual form of acquired bicuspid valve. However, in general, bacterial endocarditis occurs less commonly in these valves than calcific disease.

Summary and Conclusions

Five cases of acquired bicuspid aortic valve are described. In each case the conjoined cusp presented a markedly retracted, horizontal raphe deep in the sinus of Valsalva. This low and unusual position of the raphe is evidently due to downward displacement of its commissural attachment.

The lesions are inflammatory in origin and in all probability due to rheumatic fever. The evidence for a rheumatic origin rests on the morphology of the commissural raphe and also on the presence of stigmas of rheumatic fever, both in the aortic valve and elsewhere in the heart.

Calcific disease of the aortic valve was present in 4 of the 5 cases and 3 of these showed aortic stenosis.

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[*Illustrations follow*]

DESCRIPTION OF PLATE

PLATE 42

FIG. 1. Case 1, a white male, 59 years old, with an acquired bicuspid aortic valve. The raphe at commissure B is markedly retracted and lies at the bottom of the sinus of Valsalva, just above the attachment of the aortic valve.

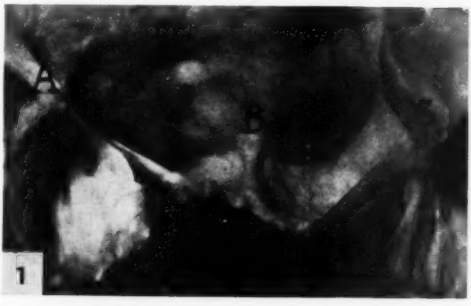
In this and succeeding figures, A, B and C represent the left-right, the right-noncoronary, and left-noncoronary commissures respectively, while lc and rc indicate the ostia of the left and right coronary arteries respectively.

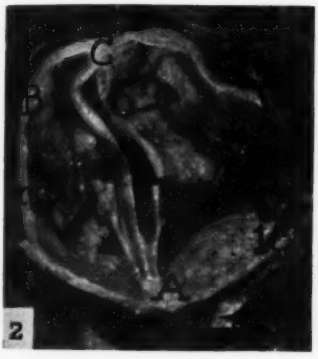
FIG. 2. Case 2, a white male, 64 years old, with an acquired bicuspid aortic valve, the seat of calcific disease with stenosis. The raphe at commissure B is retracted, horizontal and calcified.

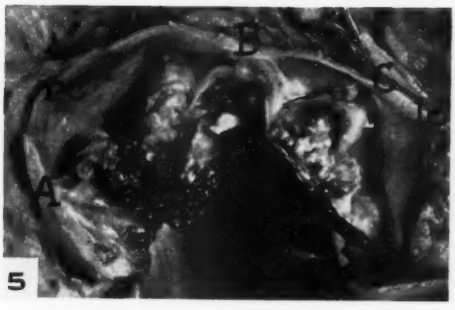
FIG. 3. Case 3, an acquired bicuspid aortic valve, the seat of calcific disease. The raphe at commissure B consists of a calcified ridge of peculiar shape situated deep in the sinus of Valsalva.

FIG. 4. Case 4, a white male, 63 years old, with calcific stenosis of an acquired bicuspid aortic valve. At commissure B is a low, prominent, horizontal raphe which is calcified.

FIG. 6. Case 5, a white male, 64 years old, with calcific stenosis of an acquired bicuspid aortic valve. At commissure C is a calcified horizontal raphe, situated just above the attachment of the aortic valve.







Koletsky

Bicuspid Aortic Valves

BACTERIAL ENDOCARDITIS DUE TO CLOSTRIDIUM WELCHII *

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(From the Department of Pathology, McGill University, Montreal, Quebec)

While bacterial endocarditis is most frequently caused by streptococcal, staphylococcal, or pneumococcal infections, other organisms in great variety have been reported as etiological agents in small numbers of cases. Shiling,[1] in 1939, reviewed the cases of bacterial endocarditis in which he felt there was sufficient clinical, pathological and bacteriological evidence to establish the causative organism. He was able to compile a list of twenty-six different bacteria as the offending organisms in bacterial endocarditis and added two additional organisms which had occurred in cases of his own. Neither in Shiling's review of the literature nor in a search of the literature since that time was there encountered a single case in which endocarditis was caused by *Clostridium welchii* (*perfringens*). It is true that Janbon and associates[2, 3] reported three cases of *Cl. welchii* septicaemia, associated with "endomyocarditis" in two cases and endocarditis in another, but the diagnosis of cardiac involvement was made on the finding of positive blood cultures, changing heart murmurs and myocardial failure alone. In none of these cases was an autopsy performed to confirm the clinical diagnosis. The purpose of the present communication is to report a case in which the diagnosis of acute bacterial endocarditis due to *Cl. welchii* was confirmed by post-mortem examination.

REPORT OF CASE

V. H., a married woman, 34 years old, was admitted to the gynaecological service of the Montreal Maternity Hospital on January 3, 1941, complaining of menorrhagia. She had a previous history of Sydenham's chorea at 11 years of age, with subsequent heart damage which had not been incapacitating at any time. She had a normal menstrual history until August, 1940, when she began to have a change in the character of her menstrual periods. There were no other significant findings in her personal or family history.

On admission, the temperature was 99° F.; pulse, 88; respiration, 20; blood pressure, 182/106. There were harsh apical presystolic and mid-diastolic murmurs and similar murmurs over the left sternal border. The posterior lip of the cervix was thick and hard. A specimen of the cervix was reported as carcinoma solidum.

On January 6th radium was inserted into the cervix and this was removed on January 7th. One hour after the removal of the radium the temperature was 101° F. The patient continued thereafter to have a daily fever varying from 102° to 104° F. associated with chills. Sulphanilamide was administered commencing on the second day of the fever. A swab of the cervix taken on January 14th, 1 week after removal of the radium, was reported positive for *Cl. welchii*. Blood taken on

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† James Douglas Research Fellow in Pathology.

January 16th yielded a pure culture of *Cl. welchii*. At this time jaundice was noticed; the skin was hot and flushed, and tender petechiae were present on the right palm and fingers. There was a harsh basal systolic and a high-pitched prolonged diastolic murmur and it was thought that the patient had septicaemia and endocarditis. Repeated plasma and whole blood transfusions were given. Administration of sulphathiazole was substituted for the sulphanilamide therapy on January 17th. On January 25th the spleen and liver became palpable for the first time and the spleen was tender on pressure. At this time 20,000 units of anti-gas-gangrene serum were given followed by another 5,000 units on January 29th. On February 1st, signs and symptoms of occlusion of a large artery to the left lower extremity appeared. Gangrene of the left leg developed which necessitated amputation on February 19th. Abscesses were found in the necrotic anterior tibial muscles from which *Cl. welchii* was grown in pure culture. The patient became sensitized following the injections of anti-gas-gangrene serum and no further antiserum could be given, as attempts to desensitize the patient failed. *Cl. welchii* was grown in pure culture on two additional occasions from blood taken on February 21st and March 5th. Numerous transfusions were given but the patient became steadily weaker and died on March 10th, 9 weeks after the first symptoms of infection.

Gross Examination

A complete post-mortem examination (no. 10843), excepting the contents of the cranial cavity, was performed 7 hours after death. In the following summary all of the pertinent findings are included.

The heart, weighing 525 gm., showed hypertrophy and dilatation of both right and left sides. The free margins of the mitral valve leaflets were firm and thick and the chordae tendineae of the mitral valve were shortened, thickened and inserted into the mitral valve by broad attachments. There was a rough patch of thickened endocardium on the posterior wall of the left auricle. The aortic valve cusps were stiff and opaque with markedly thickened margins. Between the noncoronary and the left coronary cusps of the aortic valve and extending over their adjacent ventricular surfaces was a brown, friable, mulberrylike vegetation over an area of 2 by 3 cm. There was ulceration and perforation of the noncoronary cusp of the aortic valve (Fig. 1).

The lungs were heavy and hyperemic. The bronchi contained thick bloody mucus and the mucosa was congested. An embolus partly filled the lumen of the superior mesenteric artery. A segment of the terminal portion of the jejunum, about 1 foot in length, was hyperemic, oedematous and covered by a thin fibrinous exudate. The lower pole of the spleen, the adjacent stomach, colon and omentum, joined by adhesions, formed the walls of an abscess cavity containing thick greenish yellow pus. The spleen was enlarged, weighing 290 gm. The capsular surface presented many yellow, irregular, bulging areas measuring up to 2 cm. in diameter, some of which were firm, corresponding to underlying areas of infarction. Some, however, were fluctuant due to abscess formation. The right kidney presented two firm yellow areas of infarction

in the cortex, 1.5 cm. in diameter, and an abscess of similar size. In the cervix there were two deep lateral fissures. The mucous surfaces of the cervical canal and uterus were opaque, white and finely granular, covered with a film of purulent-appearing fluid. Almost the whole length of the left common iliac artery was filled with a thrombus which at one point was soft and purulent. Similar material was present in the right hypogastric artery. There was a recent midthigh amputation of the left lower extremity. The dorsum of the right foot was somewhat blue and desiccated in appearance. In none of the tissues examined was there any grossly detectable gas formation.

Microscopical Examination

The heart was studied histologically in a number of sections taken to include myocardium, endocardium and the mitral and aortic valve cusps. There was a hyaline connective tissue thickening of the aortic valve cusp with many well formed arterioles in its thickened base. On the aortic surface of the valve and towards the free margin of the cusp there was a layer of young fibrous connective tissue covered by endothelium. On the ventricular surface of the valve cusp, extending from the free margin to the base, was a thick mass of fibrin and platelets containing many degenerating neutrophils and a few minute areas of coarsely granular calcium deposit. Beneath this vegetation, especially at the base of the valve cusp and in the adjacent myocardium, there was a heavy infiltration of polymorphonuclear leukocytes, lymphocytes and large mononuclear cells, associated with marked vascular dilatation. The deeper layers of the vegetation showed some organization. In a section of the aortic valve stained by Glynn's[4] method for the demonstration of Gram-positive and Gram-negative organisms, great numbers of large Gram-positive bacilli possessing the morphology of *Cl. welchii* were found throughout the vegetation and were especially abundant in its deeper parts (Fig. 2). A careful search with the oil immersion lens failed to reveal organisms of any other morphology. The mitral valve presented marked hyaline fibrous connective tissue thickening. In the base of the valve there were many thick-walled muscular arteries. The endocardium of the left auricle was thickened and sparsely infiltrated with small round cells. There were numerous areas of perivascular fibrosis in the heart muscle, in some of which definite Aschoff cells could be distinguished.

Both lungs showed chronic passive congestion with more recent hyperemia and hemorrhage into the alveoli. There was an extensive fresh central necrosis of the liver lobules. A section of the jejunum showed early coagulative necrosis. In sections of the spleen and kidney multiple

septic infarcts were found. Some of the intralobular arteries related to the renal infarcts were occluded by emboli. The infarcts in both organs were bordered by zones of hemorrhage with many pyknotic and fragmented nuclei, among which considerable numbers of degenerating polymorphonuclear leukocytes could be distinguished. The necrotic tissue in the infarcted areas showed marked disorganization of the normal architecture and was irregularly infiltrated with polymorphonuclear leukocytes in various stages of degeneration. One infarct in the spleen and one in the kidney showed complete disintegration and liquefaction of the central part which contained tissue débris and many clearly recognizable, degenerating polymorphonuclear leukocytes. Sections of the cervix showed a marked fibrosis with complete loss of epithelium. No evidence of carcinoma could be seen. In sections of the left common iliac artery, the lumen was filled with thrombotic material showing early organization of the periphery. The arterial walls showed changes ranging from a moderate mononuclear inflammatory reaction to complete destruction. Several sections of the tibial arteries taken at the time of amputation of the left leg contained an organized thrombus while others showed more recent thrombi. In addition to the sections of the aortic vegetations stained for bacteria, sections of the spleen, kidney, liver and thrombus in the left common iliac artery were also stained by Glynn's[4] method. Except for one vessel in the kidney which contained Gram-positive rods similar to those of the aortic vegetations, no bacteria were seen possessing the morphology of *Cl. welchii,* and in none of these sections were bacteria of any other morphology found on careful search.

In summary, therefore, there was found an acute bacterial endocarditis of the aortic valves which showed evidence of previous rheumatic damage. The septic infarcts of spleen and kidneys, as well as the gangrene of the jejunum and lower extremities, were interpreted as the result of septic embolism from the aortic vegetations.

Bacteriological Studies

Cultures of the heart's blood taken post-mortem yielded a pure growth of *Cl. welchii.* Pus from the perisplenic abscess gave a heavy growth of *Cl. welchii* with a few Gram-positive micrococci which were regarded as contaminants.

The second and third cultures of the blood taken during life and the culture of the heart's blood taken at autopsy were grown in media containing p-amino-benzoic acid. The organisms obtained during life from the blood and from the abscess in the amputated leg, as well as those grown post-mortem and referred to as *Cl. welchii,* were nonmotile or-

ganisms possessing the morphological and cultural characters of *Cl. welchii*. Their biochemical reactions listed below served to identify them definitely as *Cl. welchii*.

Liquefaction of gelatin..+	Glucose...acid and gas formation
Meat .....gas formation	Maltose...acid and gas formation
Litmus milk .....acid, clot, gas, stormy fermentation	Lactose...acid and gas formation
	Sucrose...acid and gas formation
Löffler's serum .....no digestion	Mannite.. negative
H_2S .....+	Salicin....negative

Discussion

Failure to find any previous autopsy reports of bacterial endocarditis due to *Cl. welchii* indicates that this is probably a rare condition. Therefore, the question whether *Cl. welchii* was really the etiological agent must be given the most careful study and all other possibilities excluded. Bacteriological studies, both clinical and post-mortem, and the pathological findings all support the conclusion that *Cl. welchii* was the etiological agent. The first blood culture taken 9 days after the onset of fever, two other blood cultures taken during life and the post-mortem blood culture yielded *Cl. welchii* and no other bacterial growth either aerobically or anaerobically. The abscesses of the amputated leg and the perisplenic abscess also yielded *Cl. welchii*. A few Gram-positive micrococci recovered from the perisplenic abscess were regarded as contaminants. Prolonged search with the oil immersion lens of sections of the aortic vegetations and other infected tissues stained for bacteria revealed only large Gram-positive rods possessing the morphology of *Cl. welchii*. Because of the gross resemblance of the aortic vegetations to those of bacterial endocarditis caused by *Streptococcus viridans*, the possibility was considered that the endocarditis had been initiated by *Str. viridans* or some other bacterial infection with subsequent implantation of *Cl. welchii* as a terminal invader. While this possibility cannot be excluded with complete certainty, there is not one whit of evidence in favour of it. There was no clinical history of an illness which could be interpreted as being due to septicaemia previous to the insertion of radium into the cervix, and a blood culture taken 9 days after the onset of clinical septicaemia yielded a pure culture of *Cl. welchii*, a result which was repeated on two further occasions during life and also post-mortem. No other pathogenic organisms were recovered in any of the bacterial cultures during life or after death. The objection might be raised that the growth of other pathogenic organisms in cultures had been inhibited by the presence of sulphanilamide or sulphathiazole in the circulating blood. This objection can be met by

pointing out that two of the three blood cultures taken during life and the one blood culture taken at autopsy were grown in media to which p-amino-benzoic acid had been added. Moreover, it is scarcely conceivable that sulphonamide therapy could completely eradicate bacterial pathogens from a large endocardial vegetation when once the organisms had become established there. Nevertheless, thorough search of histological sections of the vegetation on the aortic valve appropriately stained to demonstrate bacteria failed to reveal the presence of any bacteria other than those possessing the morphology and staining properties of *Cl. welchii*. Thus, the conclusion that *Cl. welchii* was the sole bacterial etiological agent seems inescapable.

The fact that *Cl. welchii* was growing in a vegetation bathed in oxygenated blood is not inconsistent with the known biological characters of this organism. Walbum and Reymann [5] have pointed out that a total absence of oxygen is not necessary for the growth of *Cl. welchii* and there are many recorded examples of the survival and growth of this organism in the blood stream in cases of *Cl. welchii* septicaemia. In such cases the infecting organisms represent strains of relatively low virulence and the patient may survive for a considerable time or even recover.[2, 3, 6-8] It has been known for some time that in cultures of *Cl. welchii* there may appear variants which remain constant for years, each strain possessing specific antigenic qualities. According to McGaughey,[9] Orr, Josephson, Baker and Reed [10] and Borthwick,[11] the different variants remain constant in their cultural, morphological and staining characteristics and toxin production. McGaughey stated that such variations may be responsible for the differences seen in cases of natural infection. The suppurative character of the reaction elicited by the *Cl. welchii* in the present case, resulting in abscess formation in the left lower extremity, right kidney, spleen and perisplenic region, is very unusual and is probably referable to a peculiarity of the particular strain of organism concerned in this instance. It is also peculiar that, although the organism produced gas in various culture media, none could be detected in any of the infected tissues.

The origin of the infection in this case was undoubtedly the genital tract and it seems probable that manipulation of the cervix during the insertion of radium played a part in initiating bacteremia. Russell and Roach [12] stated that in about 5.5 per cent of patients *Cl. welchii* can be grown from vaginal swabs, while Sadusk and Manahan [13] placed this figure at 8.7 per cent. Cosgrove and Barry [7] pointed out that the organisms in most of the cases must be of low virulence or more patients would die of gas bacillus infection after pelvic operations.

In view of the knowledge that rheumatic lesions of the heart valves

predispose to bacterial endocarditis due to other organisms, it appears highly probable that the presence of valvular lesions of rheumatic origin in the present case constituted an important predisposing factor in the development of endocarditis caused by *Cl. welchii*.

Summary and Conclusions

A case has been presented in which *Cl. welchii* septicaemia occurred after the insertion of radium into the cervix for treatment of carcinoma. At autopsy, an acute bacterial endocarditis of the aortic valve was found. Careful investigation led to the conclusion that *Cl. welchii* was the bacterial etiological agent. The autopsy findings also confirmed the clinical opinion that the endocarditis occurred in a heart previously damaged by rheumatic infection. In a careful search of the literature there was found no previous report of bacterial endocarditis due to *Cl. welchii* in which the diagnosis had been established by post-mortem examination.

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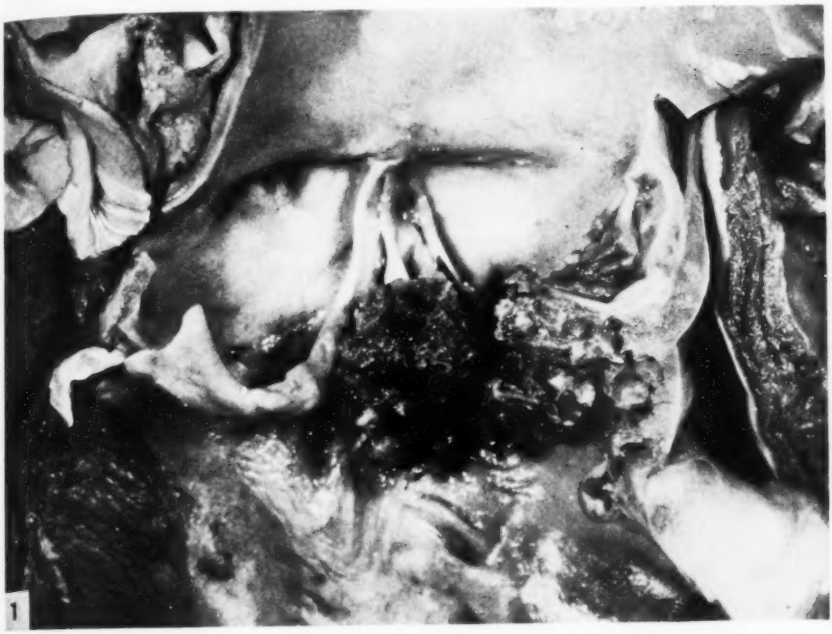
DESCRIPTION OF PLATE

PLATE 43

FIG. 1. Photograph of aortic vegetations. The left coronary cusp has been removed for section. The friable character of the vegetations may be noted as well as erosion of the noncoronary cusp and thickening of the uninvolved cusp.

FIG. 2. Photomicrograph of a section of the aortic vegetations stained by Glynn's method to demonstrate bacteria. The bacteria in this field lay deep in the vegetation, were Gram-positive and possessed the morphology of *Clostridium welchii*. × 1500.



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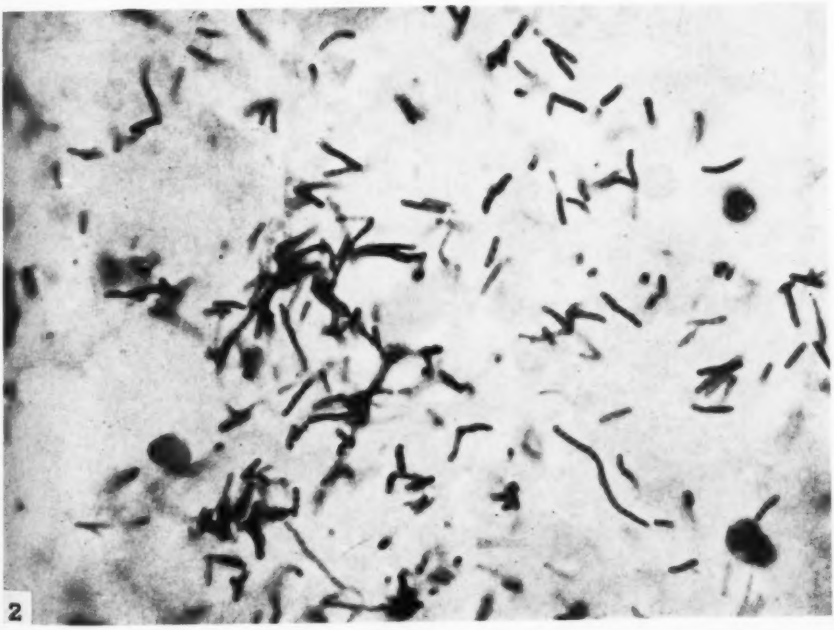

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More

Endocarditis Due to *Clostridium welchii*

HISTOCHEMICAL STUDIES ON TISSUE ENZYMES

III. A Study of the Distribution of Acid Phosphatases with Special Reference to the Nervous System *

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Introduction

Acid phosphatases with an optimum *in vitro* pH of about 5 have been found in liver and spleen,[1] in the prostate,[2-4] seminal fluid [2, 5] and urine [6] of adults, and in serum.[7, 8] Nothing is known of the function of these enzymes in liver, spleen, or kidney. However, Gutman and Gutman [5] reported that little or no acid phosphatase is present in the prostates of human beings and monkeys before puberty and that a marked increase occurs with puberty. They were able to produce an increase in the acid phosphatase content of the prostate by injection of testosterone propionate [9] and have suggested that acid phosphatase may be important in the glycolytic phases of the reproductive process. These authors also found that the acid phosphatase of the serum of normal persons is not of prostatic origin,[8] but that the marked increase in the acid phosphatase of serum which occurs in persons with metastasizing carcinoma of the prostate is due to prostatic phosphatase and is of value in the diagnosis of metastatic carcinoma of the prostate.[10] Huggins and Hodges [11] have shown that orchiectomy caused a marked drop in the elevated acid phosphatase of patients with metastasizing prostatic carcinoma.

Gomori [12] has introduced a histochemical method for the demonstration of acid phosphatases in tissue and has shown that the enzyme activity is localized in the glandular epithelium of the prostate. He has also described the distribution of acid phosphatases in other tissues, notably liver, spleen, adrenal and kidney, and has recorded the differences in distribution between acid and alkaline phosphatase.

The present study concerns the distribution of acid phosphatases in normal and neoplastic tissues of the nervous system, for which no histochemical data have hitherto been reported.[12] In the course of the work certain changes and precautions were found to be necessary to insure maximal enzyme activity. Under these conditions, acid phosphatase was observed to be more widely distributed than had previously been found [12] and a detailed description of the distribution of the enzyme in several species is given.

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METHOD

The procedure for the demonstration of acid phosphatases, as outlined by Gomori,[12] is based on the deposition of lead phosphate at the site of the enzyme activity, when a tissue section is incubated at 37° C. with an organic phosphate ester in the presence of lead ions buffered at pH 4.7.

Tissues were fixed in cold, concentrated, absolute acetone for 24 hours, using three changes of acetone. The tissues were next placed in absolute alcohol for 24 hours, followed by 24 hours in toluol, and subsequently embedded in paraffin.

Sections were cut at 10 μ and mounted on slides. These were run through two changes of xylol followed by two changes of absolute alcohol, washed rapidly in tap water and transferred to the incubating mixture warmed to 37° C.

The incubating mixture was freshly prepared for each experiment and consisted of:

12 cc. acetate buffer at pH 4.7
10 cc. lead nitrate, M./10
74 cc. distilled water
4 cc. 3.2% sodium-β-glycerophosphate

Sections were incubated for 19 to 24 hours. Sections adjacent to those stained for acid phosphatase were placed in a similar mixture of substrate which contained 0.001 to 0.01 M. sodium fluoride, which served as a control by inhibiting the enzyme activity. Upon removal, all sections were washed with 6 to 8 changes of distilled water during ½ hour to remove any excess lead. The sections were then transferred to a dilute solution of ammonium sulfide for 2 minutes, washed thoroughly in tap water, rinsed with distilled water, counterstained lightly with Harris's hematoxylin and with eosin and mounted in balsam.

In the finished section, the staining varies from light brown to black depending on the relative amounts of the enzyme present in the tissue. Control sections incubated for as long as 48 hours in the presence of as low as M./1000 fluoride gave uniformly negative results.

Yeast nucleic acid used at pH 4.7 was also found to be a suitable substrate[13] but the staining was less intense than with sodium-β-glycerophosphate under the same conditions. Glucose-1-phosphate was also used successfully.

At pH 4.7, the enzyme was unaffected by M./100 phenobarbital, M./500 sodium azide, M./500 iodo-acetic acid. M./100 magnesium ions had a slight inhibiting effect as did M./100 phlorhizin.[14]

Contrary to Gomori's observations,[12] sodium-β-glycerophosphate was found to be a suitable substrate provided too high a concentration was avoided. A comparative study using adjacent sections and the same concentration of substrate showed that much less staining was obtained in unit time with a mixture containing 52% α-glycerophosphate as substrate than with pure β-glycerophosphate. Optimal staining was obtained at pH 4.7 to 5.1.

Unless conditions are adequately controlled, the poor penetrating power of the acetone used as a fixative may lead to erratic results. To determine the effective penetrating power of acetone, sections from several blocks of tissue were taken at increasing distances from the surface of the block. It was found that the most uniform results were obtained with sections at depths of 150 to 500 μ below the surface of the tissue in the paraffin block. At greater depths staining was much less uniform. The first few sections of tissue also showed little reaction for enzyme and as a routine procedure to obtain optimum results, the first 100 μ of tissue after levelling off the paraffin block were discarded.

The optimal time of incubation was found to be 20 to 24 hours and with tissues which were not too carefully treated, 48 hours of incubation was sometimes neces-

sary. Gomori[12] incubated most of his sections for 6 hours and only in a few instances as long as 15 hours.

Results

With these precautions, our results in general confirm the positive findings of Gomori.[12] However, because of the establishment of optimal conditions with respect to substrate, time of incubation and fixation of tissue, it has been possible to demonstrate acid phosphatase in tissues which Gomori reported as negative.

The organs of a group of freshly killed, apparently normal animals and normal and abnormal tissues removed at four routine human autopsies were studied for the presence and distribution of acid phosphatase. The animals included three rabbits, a guinea-pig and six mice. The human cases were three males, 10½, 39 and 50 years of age and a female of 75 years. The necropsies were performed from 3½ to 13 hours after death. The boy of 10½ years had an astrocytoma of the brain stem, the man 39 years old hypertension and arteriosclerosis with coronary occlusion, the man of 50 years a carcinoma of the lung which involved only the thoracic organs, and the woman a carcinoma of the colon and central lobular necrosis in the liver. A number of specimens of each organ were examined in most instances. The findings are recorded by organs or systems for the whole group. Any variations occurring in the different animals or due to the presence of a pathological condition are appended to each description.

In addition, a small group of primary and secondary tumors of the central nervous system was investigated for acid phosphatase content.

The use of the term "staining" or its synonyms in the following description refers to the demonstration of the presence of enzyme activity as carried out in the above procedure and the intensity of the stain may be taken as a rough indication of the degree of activity.

Central Nervous System. Throughout the brain and spinal cord, the neural tissue was moderately and diffusely stained. On this background, individual nerve cells stood out sharply (Figs. 1, 2 and 3), the nucleus and cytoplasm being stained most intensely. The number of stained nerve cells varied considerably from area to area. In general, it may be said that the larger neural elements were much more often and more deeply impregnated than the smaller ones. Staining of these larger elements occurred less often in the cerebral cortex, basal ganglia, thalamus and cerebellum than in the midbrain, pons, medulla and spinal cord. In the former group, the majority of nerve cells showed a more intense staining of the nuclei than of the cytoplasm, which stained moderately and diffusely. Single cells or groups of cells in the cerebral cortex, especially in the pyramidal layer (Fig. 3), were picked out sharply

through the intense staining of their cytoplasm, nucleus and parts of their dendrites and axon. In the corpus striatum, the large nerve cells were frequently intensely stained while the smaller ones stained like the majority of the cerebral cortical cells. In the thalamus, most of the cells stained moderately. In the cerebellum, the Purkinje cells and cells of the tectal nuclei were often picked out by their deep staining; the nuclei of the granule cells were intensely impregnated. In the midbrain, pons (Fig. 17), medulla and spinal cord, the nerve cells forming the various nuclei were sharply impregnated so that the various cell groups stood out boldly.

The dendrites and axons of the very deeply impregnated neural elements could at times be followed for some distance (Figs. 3 and 7). Within the larger nerve cells, particularly in the motor elements, the neurofibrils stood out sharply, and could be followed in the cell processes as well. In the white matter, the axons were sharply outlined and deeply stained (Fig. 6). The myelin sheaths were unstained. The nuclei of all three types of glia—astrocytes, microglia, and oligodendroglia—stained well but their cytoplasm was uncolored. At times, it seemed that the processes of the astrocytes stained. The nuclei of ependymal cells were deeply, and their cytoplasm more lightly stained, while in the choroidal cells both were deeply impregnated. The walls of parenchymal blood vessels were unstained except for their nuclei. This was true, as well, for the leptomeninges with the exception of the larger leptomeningeal vessels which reacted as did other blood vessels.

Peripheral Nervous System. In the cranial nerves (Figs. 4 and 5), nerve roots, peripheral nerves (as seen in muscle), a sympathetic ganglion (human) (Fig. 9) and nerves encountered in various organs, the axons stained deeply and sharply. They stood out boldly even when the surrounding tissue was unstained. The nuclei of Schwann and endoneurial cells, but not their cytoplasm, were frequently stained and the myelin sheaths remained unstained.

Heart. The muscle nuclei stained deeply and the fibers moderately or lightly (Figs. 11 and 12). In one human case in which there were areas of myocardial fibrosis, these were clearly outlined due to the lack of staining of all but the nuclei of the connective tissue cells. The pericardium and endocardium were unstained except for the light staining of the nuclei of their cells.

Arteries and Veins. The nuclei of all of the mural elements and the bodies of the smooth muscle cells were stained. The media, therefore, was clearly outlined.

Capillaries. Only the nuclei stained.

Lung. The nuclei of the alveolar lining cells were stained. Occasionally the cytoplasm of single cells in the alveolar walls was moderately stained and in rare instances the cytoplasm of all the cells stained lightly. The nuclei and cytoplasm of the bronchial epithelium stained deeply as did those of the mucous glands on occasion (Figs. 18 and 19). The smooth muscle cells took the stain less intensely, the nuclei being impregnated more deeply than the cytoplasm. The nuclei of all of the other connective tissue elements stained but their cytoplasm remained unstained. The pleura was unstained.

Trachea. The lining cells of the mucosa showed moderate staining of their nuclei and light staining of their cytoplasm. In the serous and mucous glands only the nuclei stained. The smooth muscle stained as elsewhere (see blood vessels).

Spleen. The malpighian corpuscles stained more lightly than the remainder of the parenchyma, only the nuclei of their constituent elements being stained moderately. The only exception was the spleen of the woman of 75 years, with carcinoma of the colon and a reaction following blood transfusion, in which the corpuscles stained more deeply than the pulp. In the pulp, the nuclei of the lymphocytes stained deeply, while those of the cells of the reticulum, large mononuclear elements and multinucleated cells stained moderately. Sometimes the bodies of the last stained distinctly and the bodies of the large mononuclear elements lightly. The cytoplasm of the other cells was unstained. The capsule was unstained while the nuclei of the cells in the septa were stained.

Liver. The nuclei and cytoplasm of the hepatic cells were impregnated, the former more deeply (Fig. 14). The cytoplasm showed distinct coarse granulation. The nuclei of the Kupffer cells stained moderately, while their cytoplasm stained inconstantly and faintly; in some mice, however, the cytoplasm of the Kupffer cells stained well. Both the nuclei and cytoplasm of the bile duct epithelium were stained, the nuclei more deeply. In the large bile ducts there was a concentration of cytoplasmic staining near the free border of the cell. The nuclei of the connective tissue cells in the portal spaces were impregnated but the capsule was unstained.

In the woman of 75 years, the degenerating hepatic cells in the areas of central necrosis showed no staining of their cytoplasm and only irregular faint staining of their nuclei.

Pancreas. The islands of Langerhans in general stained somewhat more deeply than the acini (Fig. 16). The nuclei of the islet cells stained deeply and their cytoplasm moderately. The nuclei and cytoplasm of the acinar cells stained much less intensely but at times ap-

proached the depth of impregnation of the islet cells. The duct lining cells stained as did the acinar cells, or more lightly.

Stomach. Irregular staining of the cytoplasm of the epithelial cells and those lining the glands of the mucosa occurred. Contiguous cells often differed in their reaction, but this could not be correlated with cell types. The nuclei of these cells stained fairly regularly. In the woman of 75 years these mucosal cells all stained regularly and deeply. In the stroma, only the nuclei stained, while the muscularis showed deep staining of the nuclei and moderate staining of the bodies of its cells.

Esophagus. The stratified epithelium was chiefly unstained. Occasionally there was staining of the cytoplasm of the cells of the upper layers while their nuclei remained unstained.

Small Intestine. The cells lining the surface and the glands of the mucosa showed deep staining of their nuclei and moderate staining of their cytoplasm. In the stroma, only the nuclei stained, while the muscularis stained as did smooth muscle elsewhere.

Large Intestine. The mucosal epithelium and the cells lining its glands showed moderate staining of their nuclei and lighter staining of their cytoplasm. The mucosal stroma and the muscularis stained as elsewhere in the gastrointestinal tract.

Adrenal. The nuclei of both cortical and medullary cells stained deeply while the cytoplasm of the medullary elements was much more deeply stained than that of the cortical cells. At times, the glomerular zone of the cortex was more deeply impregnated than the rest. Cortical cells containing large amounts of lipid often appeared more lightly stained than the others due to the dispersion in their cytoplasm of positively staining, coarsely granular material.

Kidney. In the glomeruli as a rule only the nuclei stained. In some mice the epithelial cells of the glomerular loops and Bowman's capsule showed some staining of their cytoplasm. In the convoluted tubules there was moderate staining of both nuclei and cytoplasm. The descending arm of Henle's loop often showed intense staining of both nuclei and cytoplasm, while the loop and ascending arm stained similarly to the convoluted tubules or more lightly. In the collecting tubules the nuclei were deeply stained. The cytoplasm, chiefly at the cell margins, was intensely stained and this was greatest toward the lumen. In some mice the epithelial lining cells from the convoluted tubules to the ducts stained equally. In the woman of 75 years with a transfusion nephrosis there was no definite difference noted in the convoluted tubules. The ducts of Bellini exhibited deep staining of the nuclei of their cells while the cytoplasm stained lightly.

Bladder. The nuclei of the epithelial cells of the mucosa stained

moderately and the cytoplasm was unstained. Smooth muscle stained as elsewhere. The nuclei of the serosal cells stained lightly.

Prostate. The nuclei and cytoplasm of the cells lining the tubules stained very deeply (Fig. 13). The nuclei of the cells in the interstitial tissue stained moderately to deeply, but their cytoplasm was unstained except for moderate staining of the smooth muscle cells.

Testis. The cytoplasm and nuclei of both sustentacular and spermatogenic cells were stained. Of the latter the spermatogonia stained most deeply and the spermatids least. In most instances spermatozoa were unstained. In some of the mice the heads of the sperm stained deeply and occasionally the tails stained lightly. The nuclei of the interstitial cells were moderately stained while their cytoplasm remained unstained.

Epididymis. The cytoplasm and nuclei of the tubular epithelium stained moderately to deeply, the free borders of the cells being impregnated somewhat more intensely.

Seminal Vesicle. The epithelial lining showed deep staining of its nuclei and light staining of its cytoplasm.

Vas Deferens. Throughout the wall only nuclei stained, except for the smooth muscle which stained as elsewhere.

Uterus. The surface epithelial cells and cells lining the glands of the mucosa showed deep staining of their cytoplasm and nuclei (Fig. 15). The most intense cytoplasmic staining was at the free border of the cell. In the mucosal stroma only the nuclei stained. The smooth muscle stained as it did elsewhere.

Ovary. Only the nuclei stained in the capsule. The nuclei and cytoplasm of the ova stained moderately. The cytoplasm of the follicular cells stained similarly while their nuclei stained very deeply. In the ovarian stroma, the nuclei and cytoplasm stained moderately or the cytoplasm remained unstained.

Fallopian Tube. In the lining cells of the mucosa the nuclei stained very deeply and the cytoplasm moderately.

Thyroid. The nuclei of the acinar cells stained moderately and their cytoplasm lightly. The colloid material stained moderately or at times deeply.

Pituitary. All three types of cells in the anterior lobe stained, the nuclei more deeply than the cytoplasm. The intermediate zone, posterior lobe and capsule were unstained.

Parathyroid. The cytoplasm of all of the cells was stained rather lightly while the nuclei stained moderately.

Striated Muscle. The sarcolemma and muscle nuclei stained moderately. The muscle fibers stained lightly or moderately and their striations were clearly visible. At times the muscle fibers were unstained.

PRIMARY OR SECONDARY NEOPLASMS OF THE CENTRAL NERVOUS SYSTEM AND ITS MEMBRANES

Twenty-three tumors were examined. Of these 11 were gliomas, 10 meningiomas, 1 a metastatic melanoma and the last a metastatic sarcoma. These were all specimens taken for biopsy and fixed in acetone directly after their removal.

Gliomas

Astrocytoma. Three tumors were examined. The nuclei of the neoplastic astrocytes stained deeply and their cytoplasm and often their processes stained moderately. In many areas only the nuclei stained.

Glioblastoma. Three tumors of this type were examined. The nuclei of all the varieties of cells in this neoplasm stained deeply while their cytoplasm and occasionally their processes stained moderately. The necrotic areas were unstained except for nuclear fragments which stained deeply.

Medulloblastoma. One tumor was examined. The nuclei of the tumor cells stained very deeply but their cytoplasm remained unstained.

Oligodendroglioma. Three tumors were examined. One did not stain. In the other two the nuclei stained deeply while the cytoplasm was unstained or stained irregularly (Fig. 8).

Papilloma of Choroid. The nuclei of the choroidal epithelium stained deeply and the cytoplasm moderately. Only nuclei were stained in the connective tissue and blood vessel walls.

Meningioma

Ten tumors were examined. Three were negative. In the other seven the nuclei of the tumor cells stained moderately to deeply (Fig. 10). In three of the seven the cytoplasm of these cells stained moderately and remained unstained in the others.

Other Tumors

Metastatic Melanoma. The nuclei of the tumor cells stained deeply while their cytoplasm remained unstained.

Metastatic Sarcoma (Source Unknown). The nuclei and cytoplasm of the neoplastic elements stained quite heavily.

In two of the human autopsies referred to in the preceding sections tumors were encountered. One was an oat-cell carcinoma of the lung and the other a carcinoma of the colon.

Carcinoma of Lung (Oat-Cell Type). The nuclei of the tumor cells stained deeply while their cytoplasm remained unstained.

Carcinoma of Colon. The nuclei of the neoplastic elements stained deeply and their cytoplasm moderately.

Discussion

Evidence that the histochemical method for localizing acid phosphatases in tissues is specific for these enzymes has been obtained in a manner similar to that used with the histochemical method for alkaline phosphatase.[15-18] Thus when the procedure is carried out on adjacent sections of tissue without the addition of sodium-β-glycerophosphate, only tissues containing calcium are stained; when carried out in the presence of M./1000 to M./100 fluoride, a known inhibitor of acid phosphatases, complete inhibition of enzyme activity is obtained. Histochemical determination of the optimum pH for enzyme action is in agreement with the optimum pH as determined chemically. Magnesium ions have an inhibitory effect on the action of the enzyme when tested chemically[1] or histochemically. Phlorhizin was found by Beck[14] to have only a slight inhibitory effect and a slight effect was also obtained histochemically. In addition, the enzyme has been shown to be inactivated by alcohol and alcohol-fixed tissues have shown no indication of any enzyme activity.

In general it may be said that the activity of acid phosphatase is regularly demonstrable by the present method in the nuclei of cells of all tissues. This suggests that the enzyme may play a rôle in nuclear metabolism. In addition, it is encountered in the cytoplasm and processes of the cells of many organs. The nervous system is one of the most consistent sites of enzyme activity and here it is noted primarily in the nerve cell and its processes. With increasing size of the ganglion cells, their bodies show increasing acid phosphatase activity. The smallest nerve cells—the granule cells of the cerebellum and other areas—show little or no evidence of enzyme activity in their cytoplasm while the largest elements—the motor ganglion cells—exhibit the most. Axons contain considerable amounts of the enzyme and it is often demonstrable in the individual neurofibrils, while myelin sheaths are free of it. This raises the question of the possible relationship of acid phosphatase to the transmission of nervous impulses and this point is to be investigated further. While acid phosphatase activity was confined to the nuclei of glial cells, with the possible exception of astrocytic processes, the bodies of ependymal cells and more particularly of choroidal cells frequently contained the enzyme. The cytoplasm of the Schwann cells like that of the glial elements was free of it. The fact that acid phosphatase is regularly present in the cytoplasm of choroidal cells and less richly in that of ependymal cells may possibly indicate some common function, distinct from that of the glial cells in which the enzyme is absent.

In contrast, alkaline phosphatase[19] is irregularly distributed in the

nervous system and not consistently present. It is diffuse in its distribution, absent in the nerve cells and its processes, regularly present in astrocytic fibers and constantly present in the vascular endothelium of this as of other organs. Whereas acid phosphatase is as rich in the human nervous system as in that of some of the lower animals, alkaline phosphatase is comparatively sparse except in blood vessel walls.

Because of the sharp and deep impregnation of axons and, to a lesser degree, of the individual neurofibrils in the central and peripheral nervous system, the method has some value as a stain for those structures. It has the advantage of rapidity over the other methods of staining axons and neurofibrils in blocks of tissue since it can be carried out in a few days as compared with several weeks required by the other methods. The stains for axons carried out on individual sections prepared from frozen or embedded tissues are usually most distinct in tissues fixed for a number of weeks; in this instance the acid phosphatase method also has the advantage of speed. The deep impregnation of the nerve cell body and its dendrites at times resembles that seen in Golgi stains and although it is often similarly irregular it is never as complete. The axons in peripheral nerves are regularly and brilliantly demonstrated.

Gomori [12] has described the occurrence of acid phosphatase activity in a variety of organs. By the use of the present method it becomes evident that such enzyme activity is present in additional sites than those recorded. It is present not only in the splenic pulp but in the malpighian corpuscles as well, although to a lesser degree. In the liver not only are the hepatic cells positive but the lining of the biliary ducts as well. The pancreas is constantly positive, the islet cells often containing more enzyme than the acinar elements. In addition to the general nuclear acid phosphatase in the lungs, the bronchial epithelium regularly contains the enzyme. The myocardium and striated muscle are irregularly positive while smooth muscle is almost always positive. The mucosal lining throughout the gastrointestinal tract was irregularly positive for the enzyme. As Gomori [12] has pointed out, the medulla of the adrenal is rich in acid phosphatase, but the enzyme is also constantly present in the cortex although in lesser amounts. The kidney is much more often positive than has been stated. The testes are positive in man, guinea-pig, rabbit and mouse. The greatest acid phosphatase content is that in the spermatogonia and the least in the interstitial cells. The prostate is particularly rich in the enzyme, but it is also found in moderate amounts in the lining of the epididymis, seminal vesicle and vas deferens. The female genital tract is positive, the ovary and the lining of the fallopian tube and uterus containing the enzyme. A mod-

erate amount of acid phosphatase was found in the thyroid, parathyroid and anterior lobe of the pituitary.

Discrepancies between the results here recorded and those reported by previous investigators would seem to be explained by the improvements in the technic described above. It is believed that a more complete demonstration of the activity of acid phosphatase is achieved by the present method.

The function of acid phosphatase in the sites described cannot as yet be stated. It is hoped that observations made under a variety of physiological and pathological conditions may provide additional data. However, the localization of the enzyme in individual cells and structures should be of considerable value in attempting to ascribe functions to the enzyme.

As was true of alkaline phosphatase, the neoplastic elements in the tumors examined resembled their cells of origin in acid phosphatase content. For instance, in the oligodendroglioma, and as a rule in the astrocytoma and meningioma as well, only the nuclei were positive. Occasionally the cytoplasm and processes of tumor astrocytes contained the enzyme and, as was noted above, this was rarely true of normal astrocytes. Both cytoplasm and nuclei of the choroidal cells in a papilloma contained acid phosphatase as did normal choroidal cells. In contrast to the paucity of acid phosphatase in the cytoplasm of most meningiomas and its complete absence in some, alkaline phosphatase was present in considerable amounts in most of these neoplasms and was in part correlated with a tendency to calcification. No definite differences in structure that could be related to its absence could be recognized in two of the three meningiomas which contained no acid phosphatase. These two tumors were rich in alkaline phosphatase. One of the meningiomas which contained no alkaline phosphatase was positive for the acid phosphatase. One tumor which was negative for both acid and alkaline phosphatases was an angioblastic meningioma composed almost entirely of tumor vessels and containing relatively few typical arachnoid elements.

Two rather malignant tumors of the nervous system, the glioblastoma and medulloblastoma, were relatively rich in acid phosphatase. Alkaline phosphatase occurred rather irregularly in glioblastomas and was confined principally to astrocytes, whereas acid phosphatase was present abundantly in nearly all the tumor cell types. In the medulloblastoma only the nuclei, however, were positive.

In respect to the homologous reaction of tumor cells and their cells of origin, it is interesting to note that although Gomori [12] found that tumors of the pancreas and ovary were negative, as he found those or-

gans to be, two tumors originating in the bronchi were positive. This is in accord with the positive findings in normal bronchi obtained with the present method.

Summary

1. The histochemical technic of Gomori[12] for demonstrating acid phosphatases in tissues was modified to insure optimal enzyme activity. A variety of substances including enzyme poisons were used to establish the properties of these enzymes in tissue sections.

2. Using this improved technic the distribution of acid phosphatases in normal and neoplastic tissues is described. Acid phosphatase activity was found in nuclei as well as in the cytoplasm of many cells. The nervous system was found to contain large amounts of an acid phosphatase, as did both the male and female genital systems, parts of the digestive, hematopoietic, urinary, and endocrine systems.

3. A series of tumors of the nervous system was studied and the acid phosphatase content of the tumors correlated with the enzyme content of the cell types from which the tumors were derived.

4. The significance of the histochemical technic in relation to function of the enzyme in individual cells is considered.

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[*Illustrations follow*]

DESCRIPTION OF PLATES

PLATE 44

FIGS. 1 and 2. Guinea-pig. Midbrain. Deep staining of nerve cells and their processes in Figure 1 is the result of their acid phosphatase content. Figure 2 illustrates an adjacent section used as a control, in which the enzyme was inactivated by fluoride. There is no equivalent staining in this section.

FIG. 3. Human cerebrum. Frontal cortex, third cortical layer. The deep staining of some of the pyramidal nerve cells and their processes is an index of their acid phosphatase content.

FIGS. 4 and 5. Guinea-pig. Cranial nerve. Sharp and deep impregnation of axons seen in Figure 4 is an indication of their acid phosphatase content. An adjacent section shown in Figure 5, in which the enzyme has been inactivated by fluoride, reveals no comparable staining.

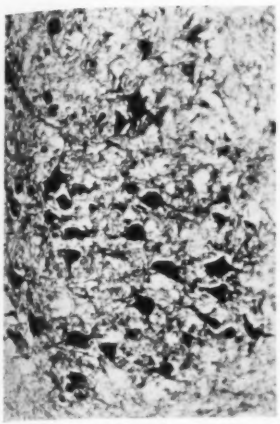
FIG. 6. Guinea-pig. Corpus striatum. The axons show a deep impregnation while chiefly the nuclei are stained in the nerve cells. The dark staining is a measure of the acid phosphatase activity.

FIG. 7. Guinea-pig. Anterior horn of spinal cord. Deep staining of motor ganglion cells demonstrates the degree of acid phosphatase activity.

FIG. 8. Oligodendroglioma. Specimen for biopsy of a human brain tumor. The nuclei of the neoplastic oligodendroglia stain deeply while their cytoplasm is unstained, indicating that acid phosphatase is entirely confined to the nuclei.

FIG. 9. Sympathetic ganglion, human. Deep impregnation of nerve cells and axons indicates the presence of acid phosphatase activity.

FIG. 10. Meningioma. Specimen for biopsy of a human intracranial tumor. Only the nuclei of the tumor cells show evidence of acid phosphatase activity.



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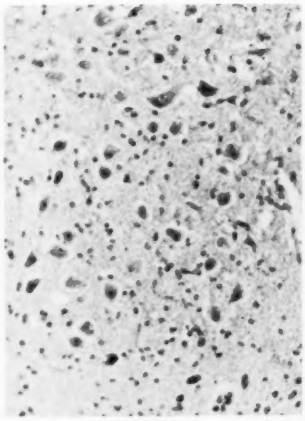

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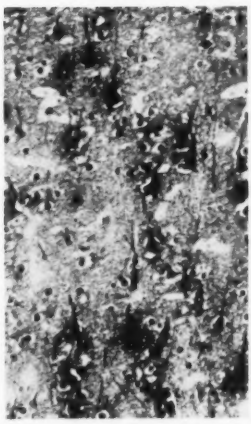

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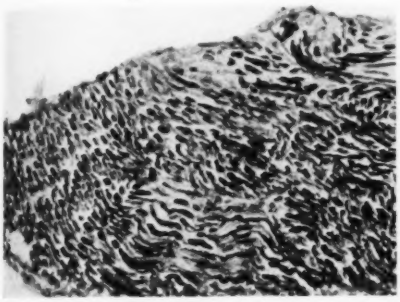

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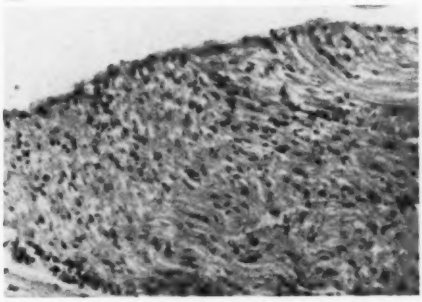

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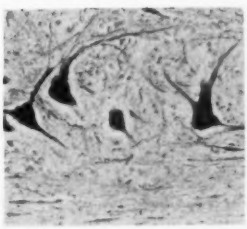

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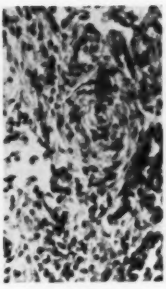

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Wolf, Kabat and Newman

Distribution of Acid Phosphatases

PLATE 45

FIGS. 11 and 12. Heart. Human myocardium. Muscle nuclei and fibers stain deeply in Figure 11, showing that acid phosphatase had been present in them. The lack of similar staining in an adjacent section of the myocardium in Figure 12 is due to the inactivation of the enzyme by fluoride.

FIG. 13. Human prostate. The presence of a considerable amount of acid phosphatase in the lining of the tubules is demonstrated by the deep staining.

FIG. 14. Human liver. The hepatic cells show evidence of their acid phosphatase content by their intense impregnation.

FIG. 15. Guinea-pig. Uterus. Dark staining of the epithelium lining the mucosal glands, of the nuclei of the cells of the stroma, and of the smooth muscle cells is the result of acid phosphatase activity.

FIG. 16. Human pancreas. Impregnation of the acinar and islet cells, the latter more deeply, is a measure of their acid phosphatase content.

FIG. 17. Guinea-pig. Pons. Deep staining of nerve cells and their processes gives evidence of acid phosphatase activity.

FIGS. 18 and 19. Guinea-pig. Lung. The bronchial epithelium, nuclei in general, and the smooth muscle of the wall of the bronchus and that of the artery, in Figure 18, are stained darkly as a result of their acid phosphatase content. The lack of similar staining, in Figure 19, is due to inactivation of the enzyme by fluoride.

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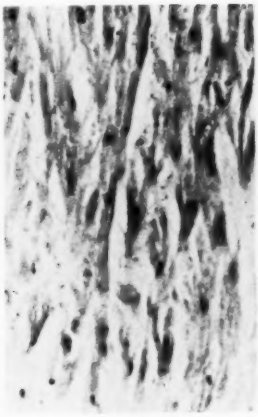

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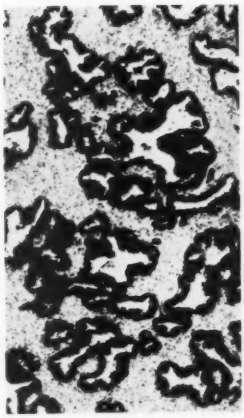

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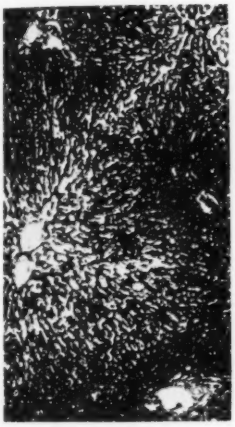

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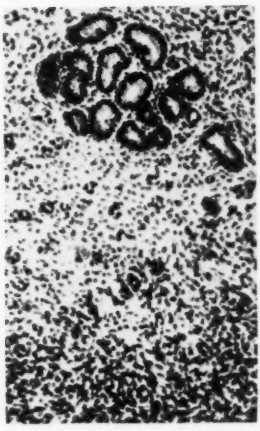

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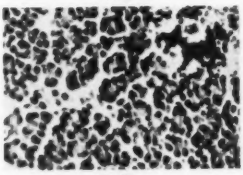

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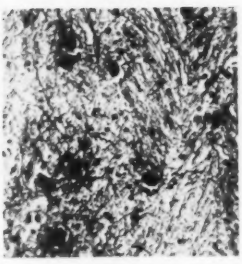

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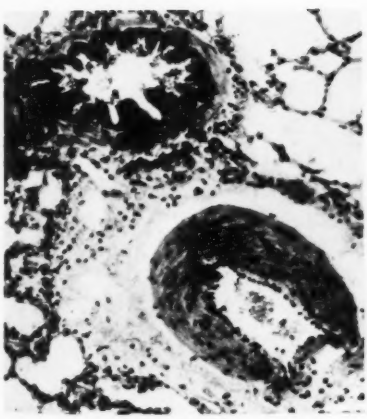

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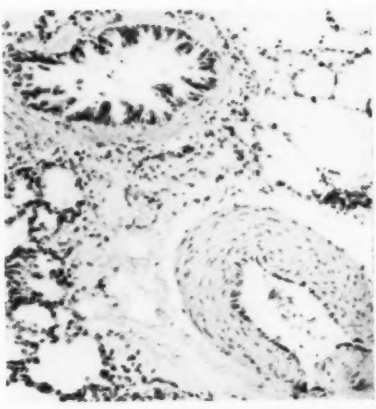

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Wolf, Kabat and Newman

Distribution of Acid Phosphatases

TUMORS OF DERMAL APPENDAGES *

I. Tumors of Sebaceous Glands by Shields Warren, M.D., and Wesley N. Warvi, M.D.
   A. Benign
   B. Malignant

II. Tumors of Sweat Glands by Olive Gates, M.D., Shields Warren, M.D., and Wesley N. Warvi, M.D. (*July*)
   A. Hypertrophy, hyperplasia and metaplasia
   B. Tumors of true sweat glands
      1. Syringoma
      2. Hydradenoma papilliferum
      3. Hydradenoma
      4. Hydradenoid carcinoma
   C. Tumors of specialized sweat glands
      1. Ciliary gland
      2. Apocrine gland
      3. Ceruminous gland
   D. Tumors ascribed to sweat glands
      1. So-called sweat gland carcinoma of breast
      2. Turban tumors
      3. Mixed tumors

III. Epithelial Cysts and Cystic Tumors of the Skin by Wesley N. Warvi, M.D., and Olive Gates, M.D. (*September*)
   A. Cysts
      1. Epidermal
      2. Epidermal—traumatic
      3. Sebaceous
      4. Sweat glands
      5. Dermoid
      6. Follicular
   B. Cystic benign tumors of familial nature, "epithelioma adenoides cysticum"
   C. Calcified cysts and calcified epithelioma

* The article which follows is the first in a series of three in which tumors of dermal appendages are described. The second and third articles will appear in the July and September numbers, respectively, of this Journal. The general plan of the series is set forth in this outline.

—*Editor*

TUMORS OF SEBACEOUS GLANDS *

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A. BENIGN

The benign tumorlike enlargements of the sebaceous glands have a histologic appearance which may not differ appreciably from that of the normal gland. There is no clear histologic distinction between so-called adenoma of sebaceous gland and the hyperplastic and hypertrophic glands.[46] The abnormality is chiefly one of increase in size, number and location of the glands (Fig. 1), with minor aberrations such as absence or atrophy of basal cells, variations in the tendency for the central cells to degenerate, or absence of ducts. Atrophy of the lesions is not uncommon; some may become fibrotic, others may entirely disappear.[26]

In pathologic enlargement of the sebaceous glands varying degrees of both hypertrophy and hyperplasia are present. Rhinophyma is a diffuse form of excessive local hypertrophy and hyperplasia of sebaceous glands of the nose, sometimes extending onto the cheeks and chin, supposedly a result of chronic inflammation.[20, 23, 53, 67] The epidermis is thickened and dotted with wide-mouthed ducts leading into enlarged sebaceous lobules full of retained secretion.

Circumscribed lesions have been described according to their clinical characteristics: (a) those occurring in old age, (b) those appearing at birth and at puberty, (c) those associated with other cutaneous and visceral abnormalities forming the syndrome of Pringle's disease.

There is a discrete glandular enlargement sometimes known as "Caspary's sebaceous adenoma" or as senile sebaceous adenoma which occurs in patients after the fourth decade, rarely earlier.[23, 59] Multiple nodules develop on the exposed parts of the face, especially the forehead. They are asymmetrically placed, small, yellowish, slightly translucent, rather flat and sometimes umbilicated, and have been described as neoplastic [50] and as hyperplastic.[27, 35, 70, 73] There is usually an associated dermatitis of the involutional senile type.[73]

The circumscribed lesion in the young patient is usually considered congenital and is frequently spoken of as a nevus. It is a single plaque-

* Because of the close anatomic relationship of meibomian and sebaceous glands we have considered tumors of these structures as a single group. A review of the tumors of meibomian glands may be found in Scheerer's paper.[61]

Received for publication, April 30, 1942.

† U. S. Public Health Service trainee.

like lesion formed of numerous papules. Histologically the normal glandular structure may be slightly altered; atrophy or focal proliferation of basal cells, mild cystic degeneration of the glands and dilatation of the ducts with keratinized material may be present.[48, 73] Jadassohn[38] first reported a form which occurs soon after birth. This is not common.[44, 45, 60] The naevus epitheliomatosus sebaceus capitis occurs on the scalp often soon after birth.[29, 54, 72] It is hairless and pitted with the orifices of the ducts. The size is variable and may reach 5 cm. in diameter. One of the few sebaceous adenomas Unna[69] was willing to accept was a congenital tumor of the scalp reported by Bock.[7] This had remained pea-sized until old age when it developed into a tumor 8 by 6 by 3 cm.

Many of the glandular enlargements on the face which occur near or at puberty are not symmetrical[33, 41] and some of them may well represent transitory functional disturbances of the gland.[39]

There are cases which do not fit into any group, such as those tumors which develop in early adult life, some of which are single, others multiple but asymmetric.[56, 58] These are probably unusual forms of the acquired senile type or the congenital type. Attempts to classify too precisely on clinical grounds nodules of the skin having similar gross structure have led to confusing distinctions.

An example of this is the controversy as to the status of adenoma sebaceum of the so-called Balzer type. Symmetrically distributed lesions over the nose, cheeks and nasolabial folds and sometimes over the chest usually appear in early childhood or at puberty.[21] Increase in size may take place several years after the first appearance.[16] They are yellow to pink and sometimes made more conspicuous by telangiectasia of the overlying skin. The arrangement and appearance of the tumors may be identical with those described as Brooke-Fordyce disease. The lesions of two similar cases reported by Balzer and Ménétrier[5] and Balzer and Grandhomme[4] as adenoma sebaceum have also been considered as Brooke-Fordyce disease (epithelioma adenoides cysticum). Contemporary criticism is inconclusive.[69] Ingels'[37] recent discussion of the relation between multiple cystic lesions of skin appendages is of interest.

The term adenoma sebaceum is usually associated with the name of Pringle,[57] who gave particular attention to its histology. Pringle's disease has come to mean a syndrome of multiple congenital abnormalities in which enlargement of sebaceous glands is constant and changes in other organs occur but are variable. The sebaceous growths are disposed symmetrically on the face, especially near the center, and are very small lobular tumors, white to yellowish brown. They appear early

in life and are often familial.[30] Von Recklinghausen's disease may be suggested by the presence of cutaneous pigmentation, nevi, papillomas and fibromas in addition to the sebaceous tumors.[11, 24, 40, 49, 62, 63, 64] In the parenchymatous organs a great variety of mixed tumors, fibromas and cysts may be found.[19, 51] Mental defects, psychoneurotic symptoms and lesions such as retinal gliomas, tuberous sclerosis and local agenesis of the brain form part of the syndrome in a large proportion of cases.[8, 9, 10, 12, 15, 17, 22, 32, 36, 47, 52] But in many cases no mental defect is obvious, and some patients are intellectually above the average.[2, 10, 34, 42, 71] Recent genetic studies by Gunther and Penrose [31] and Penrose [55] have shown with a high degree of probability "that a single dominant gene is the main causative factor—and it seems probable that 25–50 per cent of all cases are directly due to a mutation in one or another parent."

We recently had the opportunity to study the findings in an autopsy of a patient suffering from Pringle's disease. The patient was a female, 26 years old, who died of pneumothorax from so-called congenital cystic disease of the lungs. Seborrheic, flatly papular, noninflammatory lesions had been present on her nose and face since infancy and later small papillary fibromata developed over her body. Mental deficiency appeared early and grew progressively worse. Her physical condition had been good until 2 years before death. A moderate degree of dyspnea called attention to bilateral cystic disease of the lungs. Just before death there was marked albuminuria and retention of nonprotein nitrogen. At autopsy, widespread congenital abnormalities were found, most extensive in the lungs. The significant findings were:

1. Hyperplasia and hypertrophy of sebaceous glands
2. Neurofibromata of the skin
3. Congenital cystic disease of the lungs with pneumothorax on the left
4. Tuberous sclerosis associated with cystic softening of the left lenticular nucleus
5. Ovarian leiomyomata
6. Mesenchymal rests of the kidneys and retroperitoneal tissues
7. Recent hemorrhages into renal calyces

The typical sebaceous adenoma of Pringle's disease shows hyperplasia of glands with prominent basal layer of cells. Fibrosis and increased vascularity around the gland may be prominent. These lesions probably belong to the same order of growth as the congenital enlargements known as sebaceous nevi and have no other title to the term adenoma.[14] Usage has nevertheless established them as sebaceous adenomas.

The term adenoma seems to be appropriate for certain tumors of the

sebaceous glands, especially those which are solitary[14] (Fig. 2). Several adenomas have been reported from meibomian gland,[3, 6, 61, 65] as well as from other locations.[9, 43, 66] Parreira[53] reported 6 cases culled from 1282 cutaneous tumors. Two were quite typical; one other showed an independent basal cell carcinoma superficial to the adenoma and three adenomas had sudoriferous elements as well. Aisu[1] reported a case that is better designated as a hamartoma. Participation of other epithelial structures in formation of sebaceous tumors[18] is not rare in our experience and may be an explanation of some of the controversial cases.

The solitary tumorlike enlargement of the sebaceous gland we accept as adenoma. Grossly it is rounded, nonulcerated, firm, and consists of a circumscribed overgrowth of sebaceous cells producing masses larger than the usual glands and less regular in shape. These enlarged glands frequently lie close to the epidermis rather than in the midcorium (Fig. 1). Typically the picture suggests an expansile growth of the peripheral parts of the gland. As compared to the cells in the center which resemble the normal gland, those at the periphery stain more deeply, partly as a result of less and more finely divided lipoid and partly due to somewhat larger, more hyperchromatic nuclei. Mitotic figures may be absent or fairly numerous (Fig. 2).

REPORTS OF CASES

We report five tumors, none of which was diagnosed clinically as sebaceous adenoma.

Case 1

No. 30–782. C. W. B., male, 63 years of age, presented a slightly elevated nodule 2 cm. in diameter on the left ear. The duration of the tumor was not known, but it had increased in size during the previous 2 months. On examination, it was thought to involve the cartilage and it was therefore excised with the underlying cartilage. It was circumscribed and expansile, preserving the lobulated structure of the normal gland, but the individual lobules measured up to ten times the usual size. Occasional cells at the periphery of the lobule showed mitosis. The tendency of the foam cell of the normal gland to break down into fatty secretion was not noticed. The openings of the glands into the follicles were plugged with keratin and greatly dilated. Keratin was present in the distant ramifications of the dilated duct. It was evident that the gland was not secreting normally although it was actively growing. At the periphery of the primary tumor there was an early basal cell carcinoma that appeared to arise from the skin, and no connection between the two lesions was obvious in numerous sections.

Case 2

No. 35–365. A female, 53 years of age, had a flat papillary lesion on the forehead of unknown duration, measuring 0.7 by 0.3 cm. It consisted of a discrete mass of large sebaceous gland lobules lying in the midcorium. Multiple sections showed narrow, solid, ductlike connections with the skin surface.

Case 3

No. 35–1380. A female had had a verrucous, raised lesion on the right parietal region since childhood, which recently increased in size to measure 5 by 2.5 cm. It was completely excised. Hypertrophied sebaceous lobules several times the normal size made up the tumor. A moderate lymphocytic infiltration was present.

Case 4

No. 40–243. A male, 72 years of age, no history. The lesion measured 0.8 cm. in diameter and was elevated. Enlarged duct stomas were present on the surface. Histologically the tumor was made up of abnormally shaped sebaceous lobules clustered about an irregular arborescence of ducts. There was abnormal keratinization of ducts.

Case 5

No. 48909. A male, 61 years of age, no history. A papillary tumor 0.8 cm. in diameter with a slightly roughened surface was found. The main portion of the lesion was made up of a spherical mass of large atypical sebaceous gland lobules and measured 0.6 cm. in diameter. In the center of this mass there were large, irregular, ductlike structures, some filled with keratin and others with inspissated débris. There was noticeable condensation of connective tissue at the periphery of the lesion.

Malignant proliferation has been reported as frequent.[68] Two of our sebaceous carcinomas apparently developed from adenomas. Pautrier's[54] report of 10 per cent of carcinomas in 35 cases of "sebaceous nevus" is unusually high. Malignancy has been described in some cases of rhinophyma[53] and senile sebaceous overgrowths.[23]

Treatment is by destruction with carbon dioxide snow or electrodesiccation, or by excision.[23]

Anomalies of sebaceous glands are not infrequent. Some of them have no clinical significance, such as the one described by Giovannini[28] in which sebaceous glands with definite ducts are incorporated in the papillae of hair shafts. Fordyce's disease, on the other hand, merits some interest because of its common occurrence. In this condition yellow punctate lesions are found on the lip and on the buccal mucosa. Fordyce[25] noted the frequency of this condition in adults, its familial nature and its varied extent depending on the age at the time of onset. He interpreted the process as downward prolongation of epidermis with accumulation of intracellular fat and some degeneration. The resulting structures have been better described as anomalous hypertrophic sebaceous glandular elements.[67]

SUMMARY

True sebaceous adenomas are rare. We report five cases. Most so-called "adenomas" are instances of hypertrophy or hyperplasia of sebaceous glands.

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B. Malignant

The relationship of carcinoma of sebaceous glands to adenoma and to hyperplasia must be left to conjecture. The fact that many tumors persist unchanged for 10 to 20 years and then grow rapidly and ulcerate suggests malignant change in an adenoma. However, histologic evidence of such transformation is not always reliable.[4, 9, 10, 28] According to Hagedoorn,[13, 14] there is evidence that half of the adenomas of meibomian glands become malignant.

The influence of chronic irritation is suggested by the conjunction of carcinoma of sebaceous glands with rhinophyma.[34] Twort and Bottomley[42] found oleic acid very effective in stimulating growth of sebaceous cells and they produced malignant sebaceous adenomas in mice by this means. They suggested that the fatty acid breakdown products of sebaceous secretions, particularly oleic acid, make the cells more sensitive to stimulating influences, such as hormones or chronic irritation, and may be a factor influencing malignant change.

The only carcinoma that can be characterized as sebaceous is one which reproduces at least in some part the characteristics of the normal gland. There is no conclusive evidence that sebaceous carcinoma may be a product of metaplasia of basal cells and keratinized cells unrelated to the sebaceous gland or that sebaceous cells may develop keratinized or nonkeratinized cutaneous carcinomas, although in some instances the histologic picture suggests metamorphosis from one type to another.[15, 21, 37] This concept of the mutability of cell characteristics of cutaneous epithelium has complicated the study of carcinoma. Unna[43] reported one case of sebaceous carcinoma and intimated that the tumor could be recognized only in the early stages by demonstration of its origin from the gland, since he believed that the sebaceous gland structure is almost immediately lost. Masson and Géry[28] reported four tumors demonstrating gradations from basal cell carcinoma to completely differentiated sebaceous carcinoma. Grynfeltt[12] described an "epithelioma baso-sebacie" in which both basal and sebaceous elements were present. Duboucher, Montpellier and Cosset's[7] tumor, a "metatypical mixed sebaceous epithelioma," had a variety of cell types representing all cutaneous epithelial structures. Loos[25] reported a basocellular carcinoma of sebaceous gland. Morard[32] proposed four subdivisions of the main group of carcinoma of the sebaceous gland: basosebaceous, spinosebaceous, mixed metatypical and sebaceous carcinoma. Such a classification may be useful in emphasizing the variety of cell appearance found in some of these tumors. In some sebaceous carcinomas that we have seen there is a loss of differentiation in parts of the tumor and the cells may resemble keratinized epithelium or

TABLE I

Sebaceous Gland Carcinoma

Reported Cases Not Included by Beach and Severance

Author	Date	Patient		Location	Size and appearance	Duration and symptoms	Treatment and results	Remarks
		Age	Sex					
Unna[43]	1896				No data (case no. 37)			
Kren[20]	1918	74	M	Face	Pale, red, smooth, and hard; linseed size	6 years	Excised; recurred	From meibomian gland
Komoto[19]	1919	54	M	Left lower eyelid	Walnut size	6 years	Excised; recurred	From meibomian gland
Yataka[45]	1920	68	F	Eyelid	Hazelnut size	10 years	Excised; recurred twice	From meibomian gland
Akiya[1]	1920	40	F	Left upper eyelid		1 year		From meibomian gland
Masuda[29]	1922	69	M	Left upper eyelid		5 years		From meibomian gland
Pereyra[36]	1922	58	M	Left lower eyelid	Golden, nodular, hard; egg size	15 months	Excised; recurred	From meibomian gland
Letulle and de Lapersonne[24]	1923	75	M	Right lower eyelid	Golden color, hard; hazelnut size	13 years	Excised; recurred twice	From meibomian gland
Matsumoto[30]	1925	44	M	Left upper eyelid		3 years	Excised; recurred twice	From meibomian gland
Kitabori[18]	1927	59	M	Left lower eyelid	Egg size	1 year	Excised; recurred	From meibomian gland
Cicconardi[6]	1931	62	F	Scalp	Ulcerated and involved most of scalp	20 years		
Dupuy-Dutemps[8]	1932	70	M	Eyelid	Large, golden, nodular, hard	Long duration	Excised; recurred three times	Arose from adenoma
Gernez and Gasne[10]	1932	48	M	Right scapular region	Small, ulcerated, hard	7 months		Greater part was sebaceous
Milian and Brunel[31]	1933	70	M	Nose	Hyperkeratotic, hard surface	20 months		Metastases to angle of jaw
Flarer[9]	1933	55	F	Left neck	Lobulated, ulcerated; pigeon-egg size	Many months		
Pasca[35]	1934	58	F	Right upper lid	Pea size; ulcerated			From meibomian gland
Charbonnel[5]	1935	56	F	Popliteal space	In scar of burn received 50 years previously, size of palm of hand; inguinal nodes enlarged		Excised; rapid recurrence; radiotherapy of slight benefit	
Parreira[34]	1935	73	M	Nose	4 cm., ulcerated, infected	4 years	Excised; rapid recurrence; death by cranial invasion	Generalized metastases at autopsy
Parreira[34]	1935		F	Nose	Said to be similar to no. 1			Case of V. Antonia; no information
Loos[25]	1936	71	M					Specimen received without information

atypical basal epithelium. It is also true that fatty degeneration of the cells of an epidermoid carcinoma may simulate a sebaceous carcinoma, but rarely to the point of confusion with it.

The incidence of sebaceous carcinoma will be doubtful until it is generally recognized as a distinct entity. Parreira [34] gave the incidence of sebaceous carcinomas as 4.6 per cent of all cutaneous tumors. Carcinomas have been reported more often from meibomian than from other sebaceous glands.[22] Because of the rather vaguely defined criteria, we feel that conclusions drawn from the literature are of limited value. However, we have tabulated 20 cases of supposed carcinoma of sebaceous gland not included by Beach and Severance [2] in their recent review of the literature. Certain other cases were omitted because the original publications were not available.[11, 17, 33, 38, 41]

We have found 29 cases of carcinoma of the sebaceous glands among some 4000 cutaneous carcinomas. One of the tumors came from the anal region of a Great Dane. Five of our tumors, not previously reported, were included in the summary of the literature made by Beach and Severance.[2] The following discussion is based on our own experience.

There is nothing striking in the gross pathology that would suggest sebaceous carcinoma. The tumor typically develops in the middle or lower corium distinct from overlying epidermis and is often quite discrete although not encapsulated. The presence of sebaceous secretion may give a yellow color to the tumor. It is rather more apt to become infected and have a foul discharge than other carcinomas.

On the other hand, the distinctive histology labels it unmistakably. In most of the examples we have studied, the structure is that of a moderately malignant, locally invasive carcinoma. Both the cells and the pattern of growth closely resemble, or may be indistinguishable from, the normal gland. However, even in the most highly differentiated parts there are certain variations from normal structure. For example, the flattened peripheral cells of the normal gland are not present. Instead the external layer of cells tends to be slightly basophilic and less heavily vacuolated. In one very extensively infiltrating tumor there were normal appearing sebaceous glands, which were, nevertheless, an integral part of the tumor, and, nearby, finely divided strands and masses of cells of typical vacuolated sebaceous type extended widely between striated muscle fibers. However, the majority of tumors in our series have been less differentiated (Figs. 3 and 4). In these there is greater variation in size and shape of cells, the nuclei are intensely hyperchromatic, mitotic figures are more numerous and the lipoid is in finer globules or absent. The cytoplasm may be eosinophilic or

TABLE II

Sebaceous Gland Carcinoma

Number	Age	Sex	Location	Size in cm.	Ulceration	Duration (years)	Treatment	Recurrence	Metastases	Results	Remarks
21–1793							Excision				
22–787	52	F		1			Excision				
23–1088 23–1089	60	F	Temple	2.5	+	4–5	X-ray, 10/21; radium, 5/22, 6/22, 8/22, 1/23, 2/23, 3/23, 7/23	Never entirely removed		Died from extensive growth of tumor involving half of face	Carcinoma of breast(?) Carcinoma of uterus(?)
S27–581	74	M	Temple	3 x 3	+	10	Diathermy excision; radium seeds to recurrences; radium to enlarged lymph nodes	Twice	Regional nodes(?)	Died 2 yrs. after treatment, cause unknown	
S28–97 S28–98		M					Excision		Regional node		
S28–391	74	M	Temple				Excision				
29–1704	72	M	Trunk		+	>1	None		Regional nodes	Died soon after admission; autopsy performed	
30–2855	42	F	Lip	0.5							Congenital(?)
32–559	68	M	Temple	2	+	1	Excision	Once			
32–1637*	62	F	Nose			3	Cautery	Twice		No tumor 3 yrs. later	
32–1719 34–1077 39–729							Excision Radium X-ray				
34–1931	68	F	Leg			8				Died, cause unknown	Carcinoma of cervix

										Died, cause unknown	Carcinoma of cervix
35–1323*	75	M	Eyelid	2.5	+	2½	Excision				No evidence of recurrence at death
37–S–140	70	M	Face		+		Palliative x-ray 2400 r.			Died 10 weeks after admission	Extensive tumor involving upper lid, side of face, malar region
37–493*	70	F	Scalp	0.5						No tumor 4 yrs. later	
37–1534*		M	Scalp		+	½					
37–2101	54	F	Scalp	4	+	4					
37–2553	67	F	Trunk	7 x 4							
38–S–762	60	F	Arm	2						No tumor 2 years later	
38–1579	58	M	Scalp	3	+	Life					
39–1264*	63	M	Trunk	0.5		>1					
39–3142*	70	M	Forehead	1	+	1					
39–3296	71	M	Scalp								
40–782	84	F	Eyelid								
40–1875	52	F	Hand	3		4	Excision				
40–2650	66	M	Lip	2	+	1	Excision				
40–3807	77	F	Nose			3	Excision				
41–1533		M	Nose								
41–2363		M	Arm	3			Excision				
41–4337	Great Dane dog		Anus								

* Mentioned in table by Beach and Severance.[2]

basophilic and sometimes keratinized. This varied appearance often presents difficulty in diagnosing the tumor from a small specimen. The keratinization may be so marked as to suggest epidermoid carcinoma, although the absence of connection with the overlying epidermis and the architecture are not in keeping with this diagnosis. Conversely, slowly growing epidermoid carcinomas, under certain conditions, contain small fat globules as a product of degeneration, thus simulating the foam cells of sebaceous type. Ordinarily there is little confusion with typical basal cell carcinomas: the cells of sebaceous carcinoma, although sometimes basophilic and without vacuoles, are more rounded, the arrangement of the cells is less compact, peripheral palisading is absent and the architecture is not suggestive of basal cell carcinoma. It cannot be overemphasized that the typical structure of the sebaceous carcinoma is sometimes discernible in only small foci. Recurrent growths may be either more or less differentiated than the primary tumor. The two metastastic growths we have seen resembled the primary tumors.

The tumors usually occur in middle life and there is no significant difference in the incidence with which the sexes are affected. If the numerous carcinomas of the eyelid are excluded, the face and the scalp are involved with almost equal frequency and are the most important sites, but tumors occur on the trunk and extremities as well. Growth is slow, ulceration is late and even very large tumors may still be covered with intact epidermis. But there are exceptions. A few tumors ulcerate early and grow fairly rapidly.

Recurrence and metastasis are frequent problems. There was recurrence in three of our cases. One tumor recurred following excision. Two carcinomas recurred after excision and radiation. Three patients are known to be without recurrence 2, 3 and 4 years after excision of tumors. With one of the tumors which recurred later there were enlarged regional nodes. Proved metastasis to regional nodes occurred in two other cases without recurrence of the primary tumor.

In one, the tumor was a pedunculated fungating mass of friable, necrotic tissue with a foul, musty odor, lying over the fourth to sixth ribs on the right lateral aspect of the chest wall. It was said to have been present several years and had not been treated. Metastases filled the axillary nodes. The tumor and metastases were examined at autopsy. Death was due to renal insufficiency as a result of renal stone and pyelonephritis.

Prognosis is quite good if adequate excision is done at an early stage. Since metastasis is late, with few exceptions excision of recurrences may result in cure.

We have too little information to evaluate methods of treatment. All but three of the tumors in our series were excised. In one case palliative x-ray irradiation was given for a very extensive lesion of the face and the patient died soon after, apparently from the effects of the growth, although no autopsy was performed. Another tumor failed to respond to x-ray irradiation and repeated radium treatments over a period of 1½ years and the patient died as a result of the tumor involving one-half of the face. Another tumor recurred 9 months after diathermy excision but regressed after being treated with two gold seeds of 1.3 mc. each. Enlarged preauricular lymph nodes were similarly treated. This patient, who was 74 years old, died of unknown cause 2 years after the first treatment. Another carcinoma of the nasal septum and upper lip had been present 3 months when it was removed with electrocautery and an unknown amount of radium was administered to the site. There was almost immediate recurrence which was again treated with radium. One and one-half years after the first treatment there was an ulcerated, fungating, infiltrating tumor involving the nasal septum and upper lip. This was excised completely but 2 years later there was a recurrence 1.5 cm. in diameter. This was also excised. The patient, a woman 62 years old, was rather uncoöperative and was not seen again for another 2-year interval when a lesion 2 cm. in diameter was found in the same location. This was given 400 r. of high voltage x-ray irradiation and 1 month later 1000 r. of high voltage x-ray irradiation. After 2½ years there was a recurrence 2.5 cm. in diameter, involving the upper lip and causing fixation in the region of the frenum. A complete excision was again attempted and 3 years after this operation the patient was free from disease.

Although the tumors in our series have been radioresistant, we feel that radiation therapy was not given an adequate test. There is little to be found in the literature bearing on the relative efficacy of surgery and radiation. Surgical excision has been the most frequent form of treatment. Lebensohn[23] reported cure of carcinoma of a meibomian gland by radium. Manganotti[27] included carcinomas of the cutaneous appendages in his discussion of radiosensitivity and therapy without any clear-cut conclusions. Magnusson[26] stated that all superficial tumors of the skin respond in the same way to radiation regardless of their structure. This opinion is shared with reservations by van der Burg,[44] but not by Snoke.[40] Judging by Hintze's[16] report, glandular carcinomas of the skin show the same resistance as adenocarcinomas of most other organs. The effect of x-ray and radium on such carcinomas must be cauterizing rather than selective.[3]

SUMMARY

Sebaceous gland carcinoma is a pathologic entity, although often confused with basal cell or epidermoid carcinoma. We have encountered 29 cases in our laboratory. The tumor must resemble sebaceous gland in at least some portion. These carcinomas are often resistant to treatment and not infrequently metastasize. Many of them probably arise from benign growths.

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[*Illustrations follow*]

DESCRIPTION OF PLATE

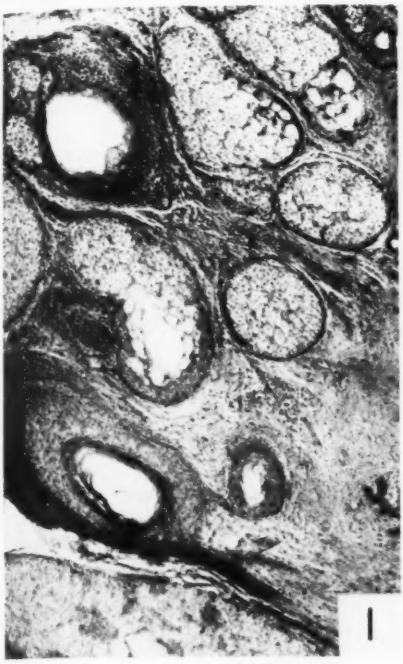
PLATE 46

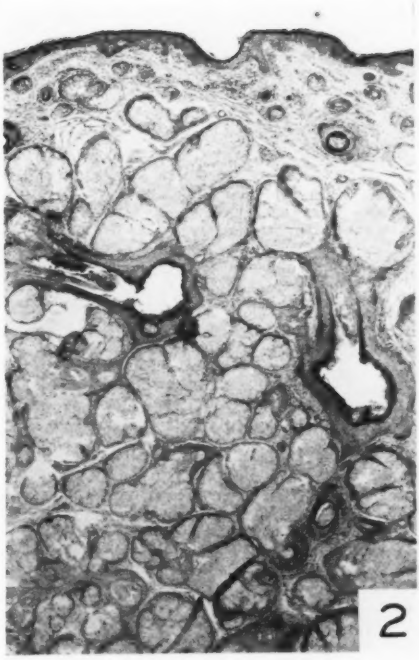
FIG. 1. Hyperplasia of sebaceous glands. Hematoxylin and eosin stain. × 50.

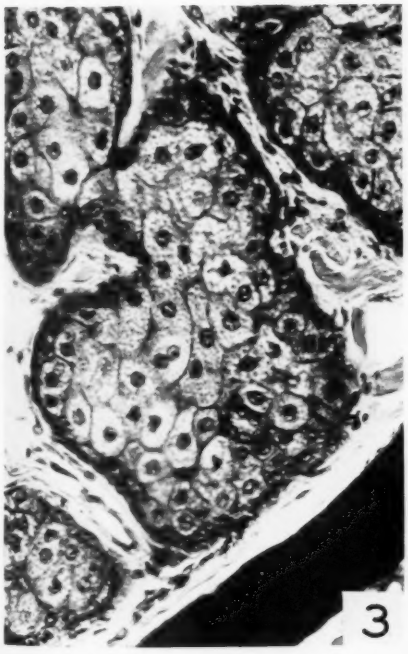
FIG. 2. Adenoma of sebaceous gland. Hematoxylin and eosin stain. × 23.

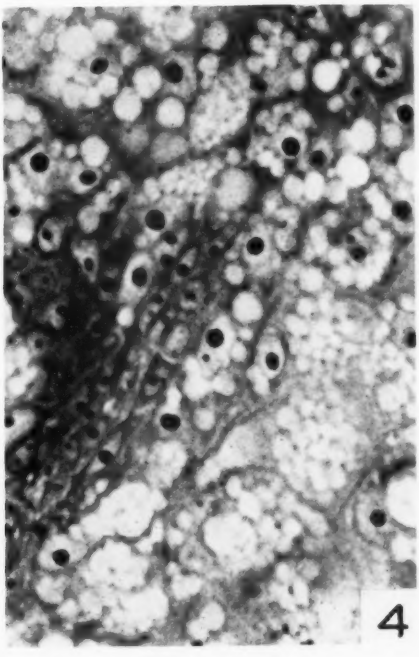
FIG. 3. Carcinoma of sebaceous gland. Well differentiated type. There is invasion about heavily stained striated muscle fiber. Phosphotungstic acid hematoxylin stain. × 265.

FIG. 4. Carcinoma of sebaceous gland. There is variation in cell size, vacuolization of cytoplasm, and dense nuclei. Phosphotungstic acid hematoxylin stain. × 900.









Warren and Warvi Tumors of Sebaceous Glands

MESOTHELIOMAS OF THE UTERINE AND TUBAL SEROSA AND THE TUNICA VAGINALIS TESTIS

Report of Four Cases *

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A degree of temerity is required in attempting to discuss neoplasms arising from endothelial and mesothelial tissues. It has even been maintained that no primary tumor of pleural endothelium or mesothelium has ever been demonstrated.[1] One obstacle in such a study is a certain disagreement as to the meaning of "endothelium" and mesothelium." Histologists are in nearly complete agreement that *endothelium* is the accepted term for the flattened cells lining the lumina of the blood vascular and lymph vascular channels, and that *mesothelium* is to be applied exclusively to the cells lining the serous cavities; *i.e.*, pleura, pericardium, peritoneum and tunica vaginalis testis. Morever, it is recognized that the vascular channels and the cellular lining of the serous cavities have independent embryological origins and anatomically are not connected.[2] Yet many writers use the term "endothelioma" for primary tumors of the serous membranes.

In this report, "mesothelioma" is used to identify primary tumors taking origin from the lining cells of the serous membranes. In 37 case reports, in medical literature, of primary tumors of the pleura, pericardium, or peritoneum, 22 are called endotheliomas, 13 are designated as mesotheliomas, and 2 as celotheliomas.

This cursory tabulation of reports of primary serous-membrane tumors from the cumulative index for the past 5 years indicates that a much larger number of tumors regarded as endotheliomas, mesotheliomas, or celotheliomas are found in the pleura than in the other serous membranes; the pericardium coming next in order of incidence, as follows: pleura, 30; pericardium, 5; peritoneum, 2. No case reports were found of tumors so named in the tunica vaginalis. One lymphangioma of the tunica vaginalis is recorded. The great majority of these reported tumors are clinically and morphologically malignant.

The four cases here reported have been encountered recently. They constitute a small group of tumors of obviously similar or identical nature, two occurring in the female pelvis and two in the tunica vaginalis of the testicle. Clinically all of these appeared to be benign in character. The histological pattern is strikingly characteristic, apparently unique and readily recognized microscopically.

* Received for publication, August 17, 1942.

I have failed to find in medical literature any accounts of tumors similar to the one here reported involving the serosa of the uterus. Several reports of tumors of the epididymis and tunica vaginalis with the characteristic histological pattern are available, but these have not been previously considered as mesotheliomas or endotheliomas, and have been variously diagnosed.

Reports of Cases

Case 1 *

H. F. (laboratory no. 936), married, white woman, age 52. Pelvic symptoms led to recognition of a tumor of the uterus. Abdominal supracervical hysterectomy was done. The patient made an uneventful recovery and has remained well.

The specimen consisted of an enlarged uterine body containing a rounded tumor mass about 7 cm. in diameter, intramural in position but extending to the serosa of the uterus. On section the greater part of the tumor appeared grossly to have the structure typical of leiomyoma, being firm and fasciculated. The serous surface presented several clear gelatinous cystic structures about 1 cm. in diameter, the surface between the cysts being somewhat roughened. The cut surface presented a distinct and peculiar zone about 8 mm. in thickness, covering that portion of the tumor immediately beneath the uterine serosa (Fig. 1). This zone appeared more homogenous and lighter in color than the remaining myomatous tumor.

Gross and microscopical examination of the tumor in its relation to the uterus made it clear that the peculiar tissue constituting the superficial zone was not confined to this area but penetrated deeply throughout the myomatous tissue. However, it did not invade the myometrium nor the endometrium. The histological features are described and discussed below. In brief, the structure was adenomatous in appearance.

Case 2 †

L. L. (laboratory no. S. J. 735), was a white woman, age 45, who for 3 years had suffered from profuse menstruation, resulting in pronounced secondary anemia. In September, 1941, subtotal hysterectomy was done by abdominal section, including the removal of both tubes and one ovary.

The body of the uterus after removal was moderately enlarged and contained multiple rounded fibromyomatous tumors. The endometrium presented a small (8 mm.), firm polyp, just above the level of the internal os. The right ovary was 3.4 cm. in its greatest diameter and contained small cysts.

* From the service of Dr. W. W. Holly, and Dr. Ralph Crumrine, pathologist.
† From the service of Dr. D. A. Harwood, and Dr. R. H. Osborne, pathologist.

The fallopian tubes were of normal appearance and size, except for the presence of a small, rounded tumor upon the wall of one tube. This was a firm, spherical nodule about 8 mm. in diameter with a granular surface and was almost white in color. Microscopically the sections presented neoplastic tissue with a glandular pattern similar to that seen in case 1. The surface was in places covered by a layer of cuboidal epithelium-like cells.

Case 3 *

L. W. (laboratory no. W. M. H. 42-360), was a white male, age 66. At examination he presented a mass in the left scrotal sac, which had been first noted 22 years before and was slowly growing. The mass was hard, smooth and of globular shape, and was apparently attached by a narrow isthmus to the lower pole of the testicle, which was otherwise normal.

The tumor was removed surgically. It was found attached to the parietal layer of the tunica vaginalis adjacent to the lower pole of the epididymis. It was free in the tunica vaginalis, which contained about 20 cc. of clear fluid, except for its attachment by a pedicle about 1 cm. in diameter. Its surface was fairly smooth, and its shape was globular, measuring approximately 2.5 cm. in diameter (Fig. 2). About 2½ months later, the operative site was healed, without symptoms or abnormal findings. Microscopically the tumor presented a neoplastic pattern practically identical with that of case 1.

Case 4 †

W. D. (laboratory no. 18-C-42-36), was a white male, age 53. For about 3 years he had noticed a small nodule in the scrotum which was slowly growing and painless. Examination revealed a small, round, hard mass apparently attached to the lower pole of the left epididymis and freely movable within the scrotum.

The tumor was removed under local anesthesia. It was attached by a broad pedicle (one-fourth of its circumference) to the epididymis, and measured 1.7 cm. in diameter. The outer surface was fairly smooth. The cut surface was firm, whitish and somewhat fibrous, and at the periphery had an apparent capsule. Microscopically the structure of this tumor was strikingly similar to that of the neoplastic tissues of the previous cases. Following removal the operative site healed without incident.

Histology

As indicated in the preceding brief case reports, these four tumors presented a striking uniformity in structure. The first impression was that of a tumor of adenomatous type. Careful study led to the conviction that the characteristic tumor cells were not epithelial in character, but were mesothelial.

* From the service of Dr. Theodore Bergman, and Dr. R. H. Osborne, pathologist.
† From the service of Dr. C. H. MacKay, and Dr. V. L. Andrews, pathologist.

The glandlike structures varied greatly in size and shape. The cells lining the acini, however, did not have the appearance of true glandular epithelium. They were markedly unequal in size and dissimilar in shape, varying from low, flat plates to a cuboidal or low-columnar form. The flat cell-forms had a tendency to take the "chain" appearance characterizing mesothelial cell membranes (Fig. 3). Many groups of cells were solid, lacking open lumina. A notable cellular characteristic was that a large proportion presented vacuolated cytoplasm, the vacuoles varying greatly in size and giving the cells a "signet-ring" appearance. These vacuoles apparently served as the origin of new glandlike acinar cavities. With the expansion of the cavity, a proliferation of the cell occurred resulting in a new acinus lined by multiple cells. Some of the rounded vacuoles and resulting lumina contained stringy or granular material which, with special stains, gave the tinctorial reaction of mucin (Fig. 4). Staining for fat showed the content of these vacuoles not to be lipid material.

The interstitial tissue framework varied in amount and was largely collagenous fibrous tissue. A moderately rich network of blood vessels was present. A striking feature was the presence of groups of lymphoid cells in certain areas, sometimes so aggregated as to suggest follicular formation (Fig. 5). Special stains for reticulum revealed an abundant network of reticular fibrils intimately related to the epithelium-like cells. Special stains for elastic fibers revealed a moderate amount of elastic tissue in the interstitial trabeculae around the cell groups.

The free surface of the tumor of the tunica vaginalis in case 3 was covered by a rather dense fibrous capsule, but in the other three tumors no definite capsule was present at the serous surface. The findings at the serous surface in the uterine tumor, case 1, were of particular significance. The surface presented multiple papillary and cystlike projections, the cysts being lined by characteristic mesothelial cells. The free surface was covered by mesothelial cells, which were manifestly hypertrophic in many areas, the cells being cuboidal or low-columnar in shape. These surface cells were seen to be continuous with the cells lining the glandlike structures in the body of the tumor through apertures into which the surface cells dipped. This finding is shown clearly in Figures 6 and 7. In the other three tumors the identity of the cell types lining the acini with the cuboidal mesothelial cells upon the tumor surfaces seems obvious. However, the demonstration of the direct connection of the surface mesothelium with the acinar cells is not so clear-cut as in the uterine tumor.

It is my impression, based upon the clinical histories of these cases

as well as the microscopical appearances, that they are essentially benign neoplasms.

Comments

A search of the literature revealed reports,[3-6] several with photomicrographic reproductions, of at least six tumors of the epididymis, of which the histories, gross descriptions and microscopical patterns indicate that they are of the same nature as the tumors here reported. These have been described under various diagnoses. One was called a cavernous lymphangioma. Three were regarded as grade I adenocarcinomas, and two as adenomas of the epididymis.

Oberndorfer[3] described his case as a walnut-sized, firm white tumor which protruded into the cavum vaginale, resting on the lower pole of the right testis. Microscopically he described the tumor "meshes" as approximately the size of seminiferous tubules of the testis. He particularly described the numerous groups of lymphocytes scattered throughout the tumor. He regarded the glandlike structure as a lymphangioma.

Thompson,[4] in his report of 13 tumors of the epididymis, included seven carcinomas. Of these seven, one was designated grade II, two as grade IV, and four were regarded as grade I adenocarcinomas. Of these last four, two were illustrated by photomicrographs which show them to be tumors of the same type as the two tumors of the tunica vaginalis in the present report. A third one was described by Thompson as being identical with the two of which photographs were shown.

Discussion

Assuming, for purposes of discussion, that these tumors constitute a group which has not heretofore been clearly recognized as such, what are the possible histogenic classifications which should be considered?

1. *Epithelial Tumors* (*Adenomas or Adenocarcinomas*). The cell morphology fails to correspond to any epithelial type with which I am acquainted. The location, and anatomical and histological relationships are inconsistent with an origin from any recognized normal epithelial structures. If, however, it should be assumed that these tumors are epithelial, are they benign adenomas or adenocarcinomas? The clinical course of all of the cases in my group, as well as those previously described, indicates a benign character. This corresponds to the histological picture, including absence of mitotic activity.

2. *Vascular Endothelial Neoplasms* (*Angio-endotheliomas*). Lymphangiomas and hemangiomas are recognized groups of vascular tumors of greater or less cellularity, but in my study of such tumors, structures

of a pattern identical to that of those here described have not been seen. And, further, the demonstrated relationship of the spaces and channels in my present group to the serous surfaces precludes angiomatous character because of the recognized histological independence of the two structures.

3. Mesonephromas. Recently attention has been called [7] to a group of tumors usually involving the ovaries which are regarded as originating from cell-rests arising in the mesonephros and recognized by the presence of structures suggesting imperfect glomeruli and Bowman's capsules. The view is held that their origin is from the embryonic mesonephros which lies in intimate relationship to the developing gonads, and that the presence of their kidneylike structures in the adult ovary is thus explained. It is admitted that there is a superficial similarity between the cellular structures here described and some of the appearances illustrated for the so-called ovarian "mesonephromas." A careful study, however, of the comparative histology fails to show any essential similarity.

4. Mesotheliomas. In view of the foregoing considerations, particularly the anatomical location of the tumors in immediate relationship to the serous membranes and the clear-cut continuity of the cells lining the acinuslike spaces with the lining mesothelial cells of the overlying serosa, it is held that these tumors should be denominated *mesotheliomas*.

That each of this small group of tumors was located in direct relationship to the generative organs suggests the possibility that the histogenic factors concerned may be related to the potentialities of the specialized mesothelium of the urogenital ridge, which in the embryo serves as the origin of the gonadal epithelial structures. It will be of interest to know whether tumors of this histological type may be found in the other serous cavities or in portions of the peritoneal cavity more remote from the urogenital ridge.

Summary and Conclusion

Four tumors of markedly similar microscopical structure, located in direct connection with the female or the male generative organs and involving their serous membranes, have been described and the fact pointed out that histologically similar tumors have been previously described, but have been variously classified.

It is concluded that these tumors represent a type not heretofore generally recognized, and that the facts presented justify the view that the characteristic cell structure is mesothelial and that the tumors may properly be considered to be mesotheliomas.

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[*Illustrations follow*]

DESCRIPTION OF PLATES

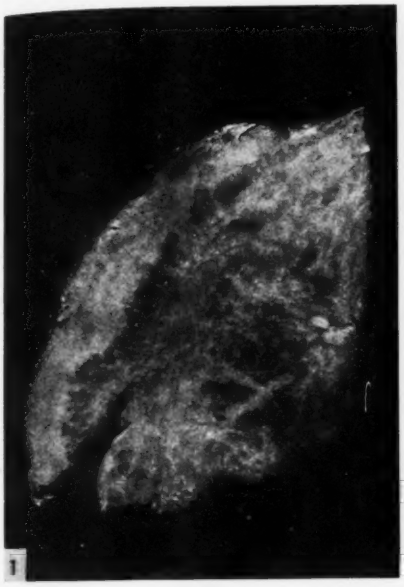
PLATE 47

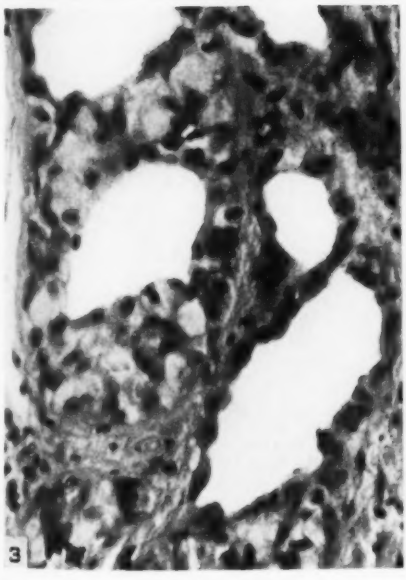
FIG. 1. Case 1. A section of the tumor of the uterus showing a light colored zone at the serous surface. The peripheral zone is mesothelial neoplastic tissue. The deeper portions are composed of interlacing myoma and mesothelioma. × 1¾.

FIG. 2. Case 3. The tumor of the tunica vaginalis showing a free rounded surface enfolded by the tunica, to which it was attached by a large pedicle. × 1¾.

FIG. 3. Case 4. Mesothelial cells surround glandlike spaces. The largest lumen is lined on one side by "chainlike" cells. Adjacent is a lumen surrounded by cells in multiple layers. Several of these cells present a "signet-ring" appearance. × 315.

FIG. 4. Case 3. Large spaces are lined by greatly flattened cells. Three collections of mucinlike material gave a characteristic coloring. Hoyer's stain. × 190.





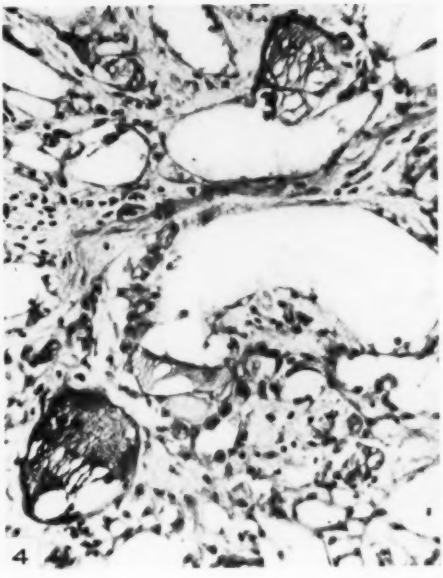


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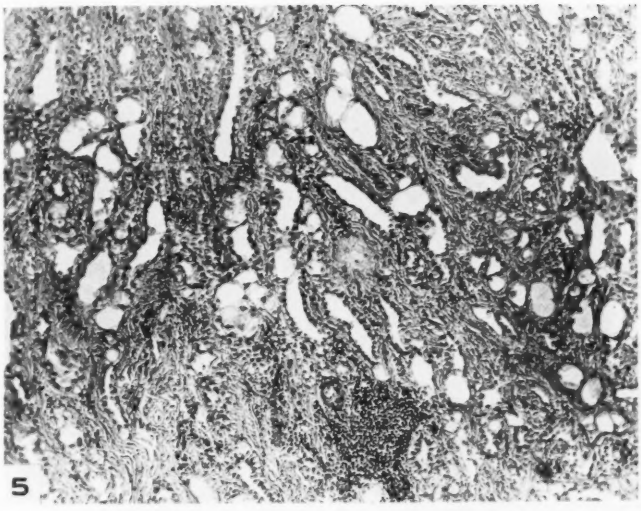
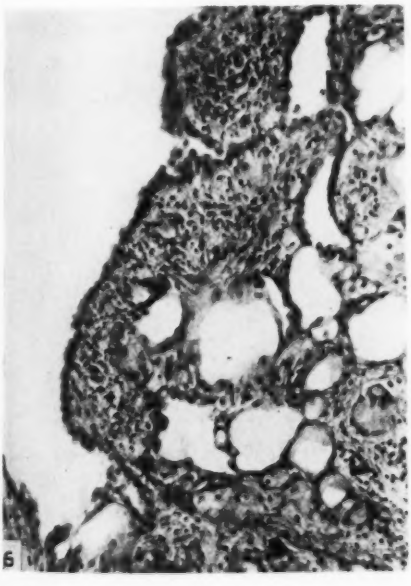
PLATE 48

FIG. 5. Case 3. Characteristic tumor pattern under low magnification showing abundant interstitial collagenous tissue and a collection of lymphocytes. × 60.

FIG. 6. Case 1. The surface of the uterine tumor covered by cuboidal mesothelial cells which are continuous through surface apertures with similar cells lining communicating spaces. × 120.

FIG. 7. Case 1. The communication of glandlike structure with surface mesothelium. The stroma near the surface contains many lymphocytes. × 140.





Evans Mesotheliomas

MYOEPITHELIAL PROLIFERATIONS IN THE HUMAN BREAST *

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The myoepithelial cell of the human breast is a smooth muscle cell which is epithelial by origin and remains on the "epithelial" side of the basement membrane. Myoepithelial cells have been described in the apocrine skin glands, in the mammary gland, in the glands of the eyelids and in the salivary glands on numerous occasions by European investigators. These are cited by Hoepke,[1] Hamperl[2] and Schultz.[3]

If one considers the mammary gland as a specialized skin gland, the presence of myoepithelial cells in this structure is more easily accepted, since histologists agree on their presence in apocrine skin glands. In a 9 mm. human embryo one can recognize an epithelial thickening forming a ridge which extends from the neck region to the groin. This has been termed the "mammillary line." Along this line the epithelial anlage of the breast is differentiated and the breast gland takes form by bud proliferation of the epithelial cells into the dermis and subdermis. The solid cell cords develop lumina and undergo cellular differentiation. The cells forming the lumen remain cuboidal and retain characteristics of their epithelial ancestry. However, the cells nearer the basement membrane become elongated and develop delicate fibrils in the cytoplasm. Such cells possess the morphology of smooth muscle cells and form what is called the myoepithelium. This is quite generally accepted, and at present there is no doubt that such cells do exist in the normal human breast (Eggeling[4]).

NORMAL MYOEPITHELIAL CELLS

In the breast the myoepithelial cells are arranged about the ducts, especially the smaller ducts that lead away from the lobules. These cells are the elongated contractile elements which are found between the epithelial cells and the basement membrane. Apocrine glands of the skin have an almost uninterrupted layer of myoepithelial cells, according to Kölliker.[5] In the eccrine glands there are spaces between the individual cells. However, the same author described thin, intercellular bridges between the epithelium and myoepithelium of both. In the breast the myoepithelial cells are isolated and appear in a proportion of about one myoepithelial cell to every six or seven true epithelial cells. The myoepithelial cells are arranged spirally about the lobular

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ducts. Less characteristically these cells may be found around acini and larger ducts. The individual cells are elongated, with a rather pale cytoplasm in which can be seen distinct, delicate fibrils as found in ordinary smooth muscle. The nucleus is rod-shaped, oval, or spindle in shape with a dense, granular chromatin material. The long axis of the cell is parallel to the basement membrane on which it appears to lie (Fig. 1). On cross section the cell frequently has a typical triangular outline, and, when crowded, the nucleus also may be triangular in shape. The base of the triangle rests on the basement membrane while the apex points toward the lumen of the duct (Fig. 2). Such cells when vesicular in nature are called basket-cells (Korbzellen). During proliferation these cells may lose their proximity to the basement membrane, but do not exceed its confines, remaining on the epithelial side.

Myoepithelium can be demonstrated in the male breast of gynecomastia when the ducts are developed. In the developed breast of the female little difficulty is encountered in its identification. In the breast in pregnancy or lactation these elements are partially obscured by the epithelial hyperplasia, but can be found on very careful scrutiny. In regard to malignant lesions of the breast, myoepithelial elements were not found in ductal carcinomas, scirrhous carcinomas, medullary carcinomas, or in malignant Paget's disease. However, the breast tissue distant from the malignant new-growth did possess myoepithelium. Myoepithelial cells are absent in breasts showing universal atrophy.

Identification of the Myoepithelial Cells

If one keeps in mind the normal position, distribution and morphology of these cells, little difficulty will be encountered in identifying them. Moreover, if one avails himself of the special staining procedures he will find a specific reaction with some dyes.

In hematoxylin and eosin preparations the myoepithelial cells have bluish black, granular nuclei and a tapering, rather large cell body with a reddish cytoplasm containing longitudinal fibrils. They are usually quite distinct from the epithelial cells except when the latter are crowded or distorted by mechanical factors, in which case the two may be confused. However, study of the cytoplasm, particularly by means of special stains, serves to distinguish them.

In sections prepared with van Gieson's stain myoepithelial cells appear brownish yellow and are usually outstanding. In addition, the van Gieson stain brings out the periductal connective tissue and demonstrates the position and course of the basement membrane. Cellular accumulations of lesser density are yellowish in color.

Probably the most specialized stain for myoepithelial cells is erythro-

sin-saffron as devised by Masson.[6] In this stain, myoepithelial cells possess a rust-red or bright red cytoplasmic substance, the shade of red depending upon the compactness of the cells.

For demonstration of the basement membrane a silver impregnation procedure serves best. A modification of the Foote method or the original Wilder method is found to be satisfactory. Demonstration of an intact basement membrane separating myoepithelial cells and the true epithelial elements from fibrous stroma or mesodermal derivatives is imperative.

The material for study is best obtained from fresh surgical specimens as soon as possible after removal. The specimen is placed immediately in Bouin's fluid for about 12 to 18 hours and then into 80 per cent alcohol. Bouin's solution was found to be the most satisfactory because it produces the least distortion and allows for good selective staining. Autopsy material may also be used but the length of time the tissue has been "dead" may produce changes which make it more difficult to study the cells accurately. However, material obtained soon after death can be fixed in the Bouin's fluid just as is the surgical material, and it has been found quite satisfactory.

Proliferation of the Myoepithelial Cells

The most interesting and significant study in regard to the myoepithelial cells is concerned with their proliferations. The myoepithelial cells of both the apocrine skin glands and of the mammary gland have the property of proliferation. Myoepithelial tumors of sudoriferous glands have been described by several European investigators as cited by Sheldon.[7] This author reported three myoepithelial tumors of the sweat glands, two of which he considered malignant.

The proliferation of myoepithelial cells usually accompanies certain other changes in the breast. Most significantly, such proliferations are found in mastopathia cystica, fibroadenomas, and glandular atrophy with hyalinization of the fibrous stroma.

Myoepithelial proliferations of the mammary gland have been reported by Günther,[8] who described the changes and also cited several other European investigators. Masson[9] gave space to myoepithelial proliferations, but this has not been carried on in standard textbooks. However, Hamperl[2] was the first to lay emphasis on myoepithelial proliferations, particularly in their relation to other lesions of the breast. He described such proliferations with special emphasis on chronic cystic mastitis and stressed the relationship of myoepithelial cells to mixed tumors of the mammary gland of the dog. Fibroleiomyoma of the breast is considered a hypothetical lesion by Foote.[10] Contrary to this,

Strong [11] and Melnick [12] each reported a case. In these instances, however, the tumor was thought to arise from the musculature of the blood vessels rather than from the myoepithelium. American literature is devoid of any references to myoepithelial proliferations of the mammary gland, in so far as could be determined.

Mastopathia Cystica

In breast tissue showing mastopathia cystica there is considerable myoepithelial proliferation which at times is so pronounced that the differential diagnosis of the lesion becomes very important. In this series 30 cases were studied; 8 cases showed myoepithelial proliferation of varying degree. These were found in persons between the ages of 50 and 70 years. Usually the proliferation takes place in the smaller ducts and their extensions within the lobules, and in such instances the myoepithelial proliferation is principally into the lumen of the duct. The earliest stage of this process is distinctly pictured in Figure 3. In this photomicrograph there can be seen six myoepithelial cells standing perpendicular to the basement membrane. The epithelial cells thereby form a small tuft which projects into the lumen. Normally the myoepithelial cells on cross section of a duct or gland have their longitudinal axes approximately parallel to the basement membrane. Evidently, then, the change in direction of the long axes of the nuclei may in this case be interpreted as one of proliferation, particularly since one can see the grouping of the cells and the progress of the cell mass toward the lumen. In some of the cystic spaces, and at times in the larger ducts in such cases, one can find a thickening of the lining cells, and this thickening can be demonstrated to consist principally of proliferated myoepithelial elements.

In Figure 4 the wall of the duct reveals a thickening of the epithelium. This portion is three to four times as thick as the remainder. Many of the cells found in this area have elongated, granular, very dark nuclei and fibrillated cytoplasm. Such cells are bright red in color in erythrosin-saffron preparations. Other cells exhibit more oval, pale, vesicular nuclei, characteristic of true ductal epithelial cells. The myoepithelial proliferation is often accompanied by an epithelial proliferation as well, and sometimes this is so far advanced that it produces actual intraluminal papillomas. On examination of such areas it is found that the papillomas are made up of two types of cells: the usual cuboidal or polyhedral type with an oval or round vesicular nucleus, and the elongated, dark spindle-shaped cells. Figure 5 shows an intraductal papilloma in which dark, elongated myoepithelial cells are the predominating elements, but there are also rounded, pale epithelial

cells, particularly about the periphery. Such proliferations are described by Hamperl [2] as epi-myoepithelial. With special stains these are found to be outstanding and no difficulty is encountered in identifying them. In such instances, with the silver impregnation methods, one can demonstrate argentophilic fibers which extend into the papillary structure, and with these particular fibers the myoepithelial cells appear to be closely associated. If the two elements are not recognized, the differences in cell morphology and intensity of staining reactions may suggest a malignant change (metaplasia) in what is really a benign papilloma. The true nature of the morphologic differences becomes apparent when silver impregnation methods reveal an intact basement membrane and special stains separate the myoepithelial cells from the epithelial cells by color differentiation as well as morphologic characteristics.

Fibroadenomata

In fibroadenomata of the breast, myoepithelial cells frequently participate in the formation of the tumor. Of 29 such cases, including fibroadenomas in the male breast, there were found 8 cases showing myoepithelial proliferation. The individuals in this group were principally between the ages of 20 to 40 years. Proliferations in this tumor of the breast, however, are frequently found to be extraluminal; that is, growth occurs away from the regular parenchyma with penetration of the stroma. In such cases there is a formation of nests or buds of proliferating myoepithelial cells which grow into the stroma. At times the proliferation is quite dense, and a very solid appearing mass of elongated, hyperchromatic nuclei is formed.

Examination of less densely packed myoepithelial proliferations shows the presence of small, round, somewhat vesicular nuclei which sometimes outline a clear space, so as to form a small lumen. These latter cells are really epithelial cells which are associated with the myoepithelial proliferation and which tend to form the acinar or glandular elements, outside of which are the myoepithelial cells. Many irregular acini are seen in Figure 6. One large area is taken up by the very cellular tumor. In this area of cellular proliferation there are found great numbers of elongated, dark, granular nuclei. Usually these are closely related to the fine, threadlike argentophilic fibers that course through the tumor. The acini are lined by pale, cuboidal cells surrounded by the myoepithelial cells. Such a unit of epithelial and myoepithelial cells is separated from others by fine, threadlike, silver-impregnated fibers.

These changes, of course, represent the advanced myoepithelial proliferations. A stage of proliferation in relation to fibroadenoma of a less marked degree is shown in Figure 7. In this area the extraluminal

myoepithelial proliferations have thus far retained their characteristic morphology and have not replaced the glandular portions of the organ to any great extent.

Fibrosis

In certain breasts, particularly those undergoing senile involution, there is found a great deal of hyalinized connective tissue stroma containing myoepithelial nests. This picture may at first glance be confusing. In spite of the fact that myoepithelial proliferations are frequently accompanied by some degree of epithelial proliferation as described above, there are found numerous nests of densely packed, hyperchromatic myoepithelial cells without any demonstrable epithelial cells being present. These nests may be large enough to take up the entire high-power microscopic field, or there may be only several closely packed hyperchromatic cells completely surrounded by a very dense hyalinized fibrous stroma.

The pathogenesis of such a change can best be appreciated after a study of the illustrations. In Figure 8 there is also some myoepithelial proliferation in the lobular duct, but this change does not take place in all instances. The lobular duct here shows a marked thickening of its wall in the upper portion of the figure with a complete absence of epithelial cells. In conjunction with this the acini are quite scarce. The left lowermost acinus shows a partial replacement of its epithelium by myoepithelium while the cell mass in contact with this acinus represents a second acinus in which the true epithelial cells are completely replaced by a proliferation of myoepithelial cells. In such instances one is able to demonstrate by silver impregnation methods an intact basement membrane which limits the cell proliferation from the surrounding fibrous stroma. It seems that in such abundant myoepithelial proliferation the epithelial glandular structures disappear, resulting in the formation of nests of the hyperchromatic, densely packed spindle cells (myoepithelial) in a dense fibrous and, at times, hyalinized stroma.

Figure 9 illustrates a process similar to that demonstrated in Figure 8. Here, however, there are isolated groups of myoepithelial cells separated one from another by connective tissue stroma, and scattered throughout this area are distorted acini. The stroma surrounding this particular remnant of the lobule is seen to be very dense and relatively acellular, and the intralobular connective tissue is similar. There are a few, irregular, isolated acini of relatively vesicular, round nuclei. In close approximation to these there are elongated and irregular nests of very dark staining, closely packed nuclei. With careful study these can be demonstrated as myoepithelial cells, as revealed by their special staining characteristics. It is quite apparent that the acini have no

normal histologic relationship, being separated from each other by dense nests of myoepithelial cells and wide bands of stromal tissue. A very significant point in the differential diagnosis of such findings is brought out in Figure 10. This high-power magnification shows small nests of irregular, markedly hyperchromatic nuclei lying in small clefts between the dense layers of stromal tissue. The similarity of this appearance to that of scirrhous carcinoma should be plainly evident.

Discussion

From the word "myoepithelial" it is apparent that the original intention was to convey the impression that these cells are muscle-epithelial cells. This had its conception in the fact that morphologically they resemble smooth muscle cells, but are more intimately associated with the usual epithelium of the glandular or functioning part of the organ; that is, these cells by position should be epithelial in nature, but by shape and structure are indistinguishable from smooth muscle cells. Consideration of their embryology leads to the conclusion that they are epithelial in origin and remain akin by position to the epithelial elements of the breast gland.

Explanations of the morphology and of the function of myoepithelial cells are not readily apparent. No definite function has been attributed to them. Much speculation has taken account of their morphologic similarity to smooth muscle and thus assigned to them the possible functions of support to the ducts or of aid in the emptying of the glands. It might be more advantageous to consider these cells in the light of their epithelial ancestry and to attribute to them some function associated with epithelial tissue. It is quite evident that they do not have a visible secretion product like the epithelial cells of the breast. Furthermore, rarely do they come in contact with the secretion within the ducts. One may postulate, therefore, that perhaps these myoepithelial cells act as receptors rather than excretors. They may be the post to which the endocrines hitch, or perhaps have an affinity for internal secretions and later deliver the same to the epithelial cells. An internal secretion (renin) has been ascribed to the eosinophilic granulations in the afibrillar myoblast of the juxtaglomerular apparatus of the kidney as described by Goormaghtigh [13] and Dunihue.[14]

At present, however, it is more important to recognize myoepithelial proliferations than to ascribe to them a specific function. In regard to the mammary gland such proliferations have been found to be entirely benign. Kölliker [5] stated that myoepithelium has the faculty of very readily changing into epithelial cells and assuming the cuboidal morphology. This represents only a "slight aberration" as these cells are

originally epithelial in origin. As such, therefore, they usually remain benign. On the other hand, Hamperl [2] traced the origin of mixed tumors of the canine breast to myoepithelium. Allen [15] believed in a similar occurrence but gave the epithelium and not the myoepithelium as the origin. In these instances the tumors are more apt to be of the malignant type since the myoepithelial cells in such cases are "totipotent." Certainly in such cases there is less differentiation, and malignant changes naturally would be more frequent. A malignant myoepithelioma was reported by Gaudier, Grandclaude and Lambret.[16] This impression was based on cytology alone. The tumor was encapsulated grossly and was clinically benign. Therefore, it is dubious that the tumor was malignant in the usual sense of the word.

Myoepithelial proliferations may be confused with true malignant changes. As found in cystic mastopathia, proliferations of the myoepithelium frequently consist of irregular thickening of the ductal epithelium, intraluminal papillomatous formations, or proliferating buds of epithelium-like cells penetrating into the stroma. In myoepithelial proliferations in lesions known as fibroadenomatosis there is a prominent stromal infiltration by hyperchromatic, elongated cells. In addition to such cells, the usual epithelial cells are at times found forming small, irregular acini, but these structures are always sharply differentiated from the true stromal tissue by the presence of an intact basement membrane. In hyalinized fibrosis of the breast there are found nests of myoepithelial cells which have survived. Such cells are usually irregular in outline, elongated and hyperchromatic. The nests of these cells are also irregular and are found in the clefts of a very dense connective tissue. In all of these cases, therefore, the elongated, hyperchromatic type of myoepithelial cell, which is found penetrating into the stroma and in isolated nests in the dense connective tissue stroma, may erroneously be interpreted as an aberrant form of the usual epithelial cell. Such aberrations usually suggest the possibility of malignancy. Here, however, they do not indicate malignancy, and such cells may be recognized as myoepithelial if their morphology is closely scrutinized and special stains are used. Such proliferations are always enclosed by an intact membrane which separates them from the mesodermal derivatives. Such lesions have been diagnosed as metaplasia, as precancerous, or as malignant "degeneration." This is evident if one considers those patients with so-called carcinosarcoma (Saphir and Vass [17]), microscopically diagnosed as such, who live in good health for many years. From a study of photomicrographs in such cases and from the clinical courses of the patients, it may be concluded that some of these tumors

were myoepithelial in nature. In another article, Saphir and Parker [18] described an intracystic papilloma classified as group III papilloma or "transitional cell type." Such a lesion possesses elongated, spindle-shaped cells, and "may possess an inert degree of malignancy." However, they add that with simple mastectomy the prognosis is good. In the light of the study here reported one might interpret the "transitional cell" papilloma as a benign myoepithelial papilloma. Furthermore, under the title of "Borderline Breast Tumors," Bloodgood [19] presented cases which lived in good health for long periods even though the lesion was suspected of being maligant. Upon comparing his photomicrographs with those presented here, the similarity becomes apparent. Bloodgood, however, did not describe these proliferations as myoepithelial in origin.

For these reasons it is very important in the study of neoplasms of the breast to identify myoepithelial tumors and to allot to such their good prognosis. It is also important not to confuse the myoepithelial tumors with malignant transformations of intraductal papillomas or with carcinosarcoma, scirrhous carcinoma, or malignant transformation in so-called fibroadenomatosis.

Summary

1. Myoepithelial cells of the mammary gland possess the faculty of proliferation, either alone or in conjunction with the usual epithelium, especially in breasts showing mastopathia cystica and fibroadenomatosis.

2. Myoepithelial cells have the power of survival and proliferation in senile involution and fibrosis of the breast.

3. In so far as is now known, proliferations of the myoepithelial cells are benign as long as they retain the characteristics of their epithelial ancestry but may become malignant when forming derivatives of the type usually ascribed to mesoderm.

4. Borderline or suspicious breast lesions should be carefully studied for the presence of myoepithelial elements. If the proliferations are found to be myoepithelial, such lesions are benign and should be distinguished as such.

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DESCRIPTION OF PLATES

PLATE 49

FIG. 1. Adenoma of the breast from a woman, 60 years old. The longitudinal, spindle-shaped dark nuclei at the base of the epithelial cells and in close proximity to the basement membrane belong to the myoepithelial cells. Hematoxylin and eosin stain. × 585.

FIG. 2. Fibrocystic disease of the breast in a colored female, 55 years old. In this illustration the myoepithelial cells are seen in cross section, thus demonstrating their characteristic triangular shape when so cut. Myoepithelial cells are more abundant than usual. Hematoxylin and eosin stain. × 585.

FIG. 3. A cross section of a small duct from the breast of a woman, 67 years old, with mastopathia cystica and Paget's disease. At the base of the small cellular tuft which protrudes into the lumen from the lower left wall, the elongated dark nuclei standing on end belong to the myoepithelial cells. This is the beginning of an epi-myoepithelial intraductal papilloma. Hematoxylin and eosin stain. × 585.

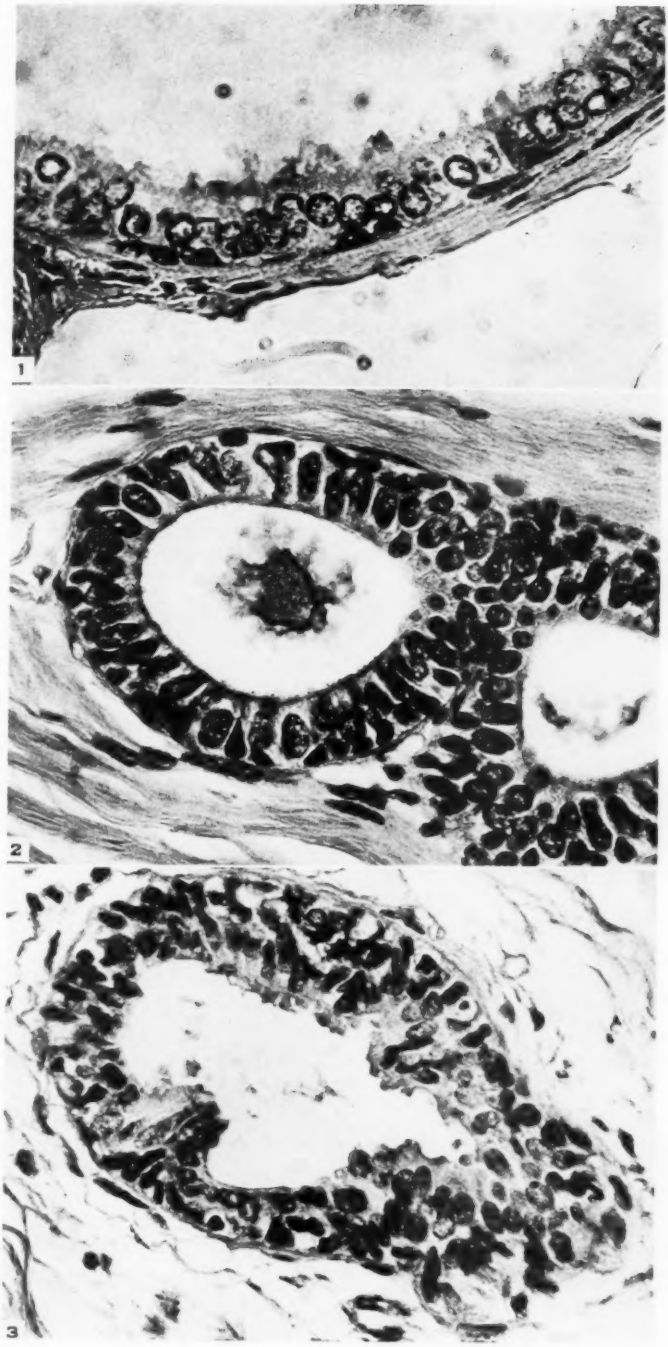


PLATE 50

FIG. 4. Fibrocystic disease of the breast in a colored female, 52 years old. The illustration includes a portion of the wall of a large duct with stasis and dilatation. In the portion of the thickened epithelium the elongated, deeply stained nuclei, which are here perpendicular to the basement membrane, belong to myoepithelial cells. The cytoplasm of these cells clearly shows fibrils. The basement membrane was demonstrated to be intact. Hematoxylin and eosin stain. × 655.

FIG. 5. Fibrous mastopathy from a woman, 30 years old. An epi-myoepithelial proliferation has produced a papilloma within a duct. The cells composing the papilloma are chiefly myoepithelial, as is shown by the dark, elongated nuclei. There is an occasional spherical, vesicular epithelial nucleus. Hematoxylin and eosin stain. × 75.

FIG. 6. Fibroadenoma of the breast in a middle-aged woman. The entire field is a tumor mass of myoepithelial cells accompanying which are fewer epithelial cells. The irregularly outlined, small, clear spaces are actually acini formed by distorted epithelial cells. Otherwise the cells are myoepithelial. Hematoxylin and eosin stain. × 75.

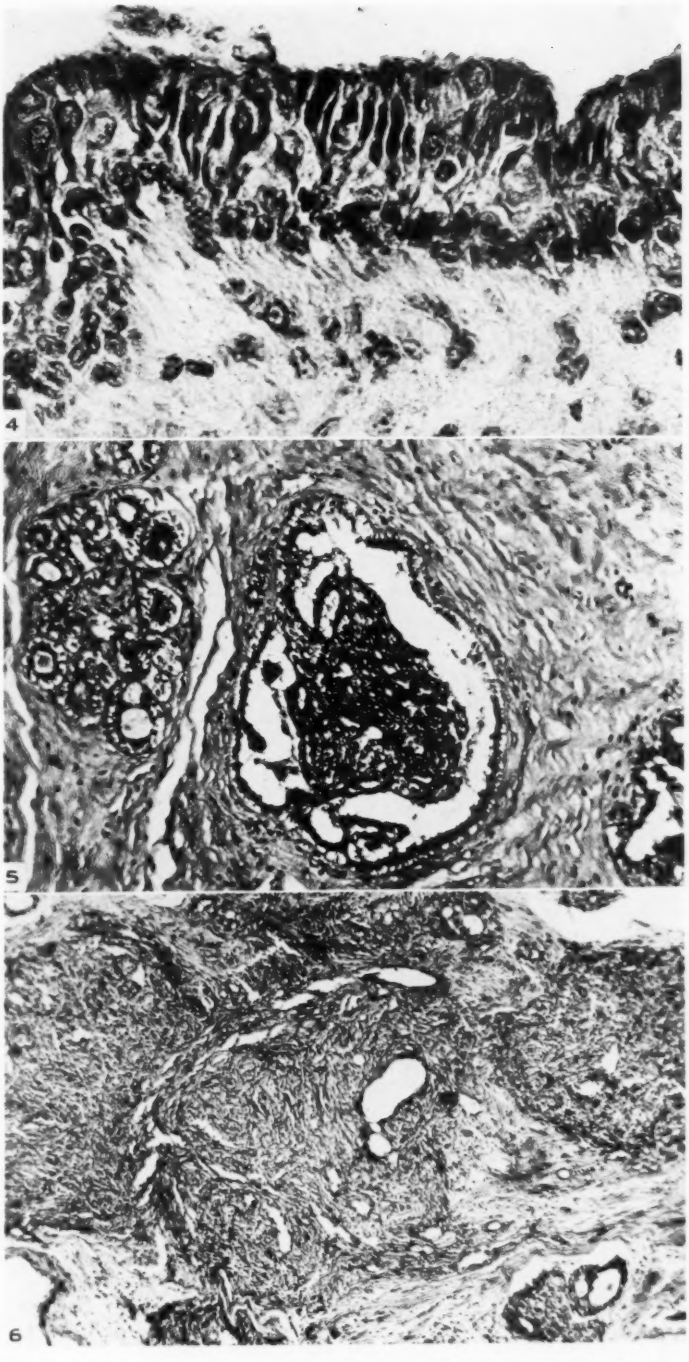
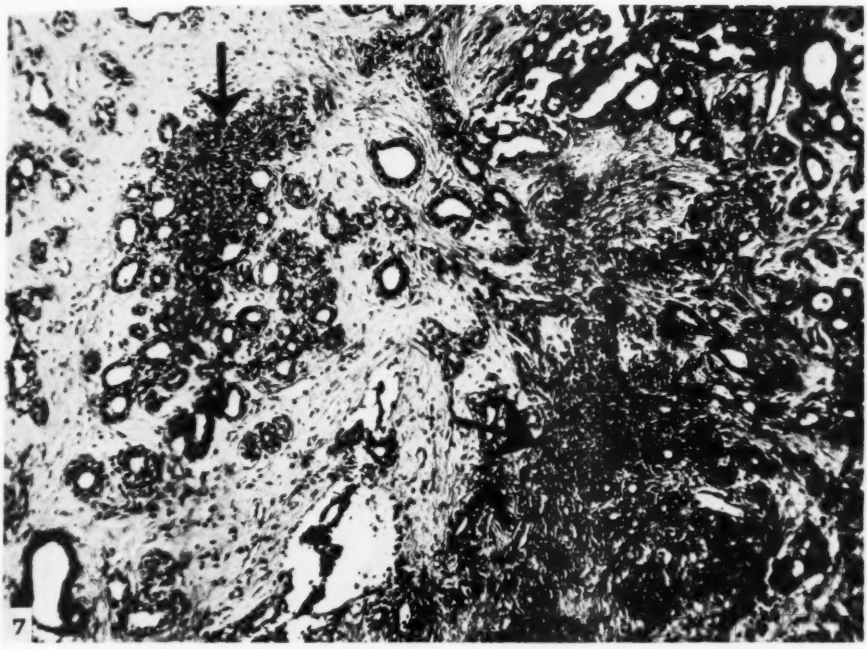


PLATE 51

FIG. 7. This field was made from the breast of a colored woman, 28 years of age. Upon this a microscopic diagnosis of adenofibroma had been made. Here there are shown extensive, yet distinct, myoepithelial proliferations in the stromal tissue from which they are separated by the usual basement membrane, now distorted and tortuous but intact throughout. Hematoxylin and eosin stain. × 90.

FIG. 8. Fibrosis of the breast with dense and relatively acellular stroma. Only the remnants of a lobule remain. The upper portion of the lobular duct reveals a significant myoepithelial proliferation. The dark mass of cells above the lowermost acinus represents a second acinus entirely replaced by myoepithelial cells. Hematoxylin and eosin stain. × 90.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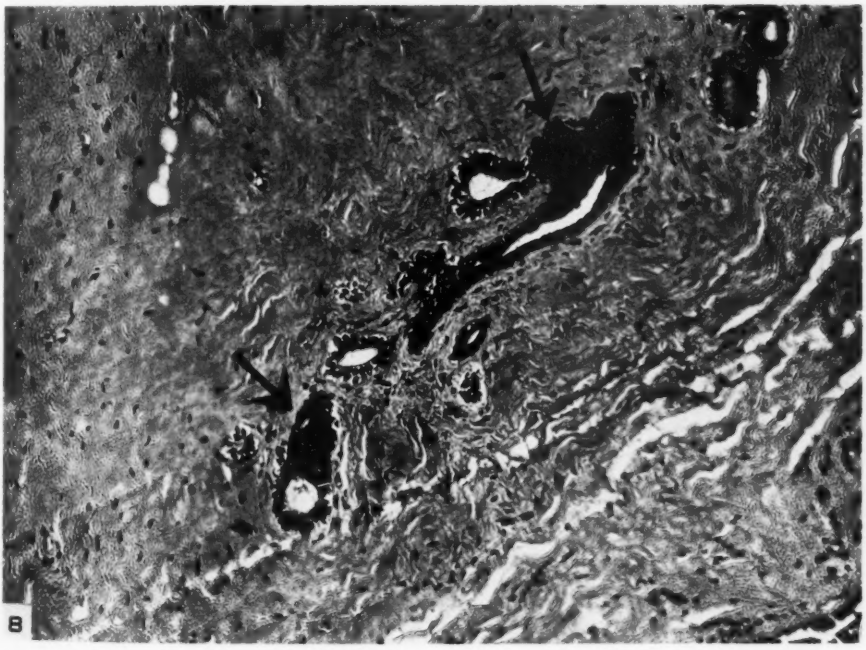
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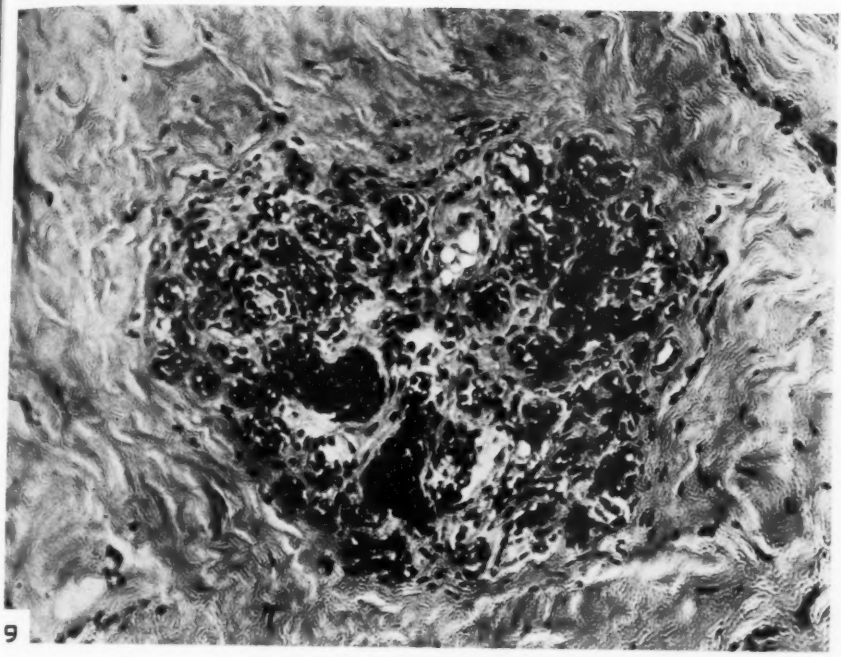
PLATE 52

FIG 9. Intralobular fibrosis and epithelial atrophy of the mammary gland. Irregular nests of elongated hyperchromatic cells have replaced much of the original lobule. These cells are myoepithelial. Hematoxylin and eosin stain. × 140.

FIG. 10. This illustration demonstrates the difference in morphology between acinar epithelium and an elongated group of myoepithelial cells. Such hyperchromatic, elongated, and at times irregular cells may be confused with scirrhous carcinoma when they are found in fibrous stroma after total loss of epithelial cells. Hematoxylin and eosin stain. × 785.



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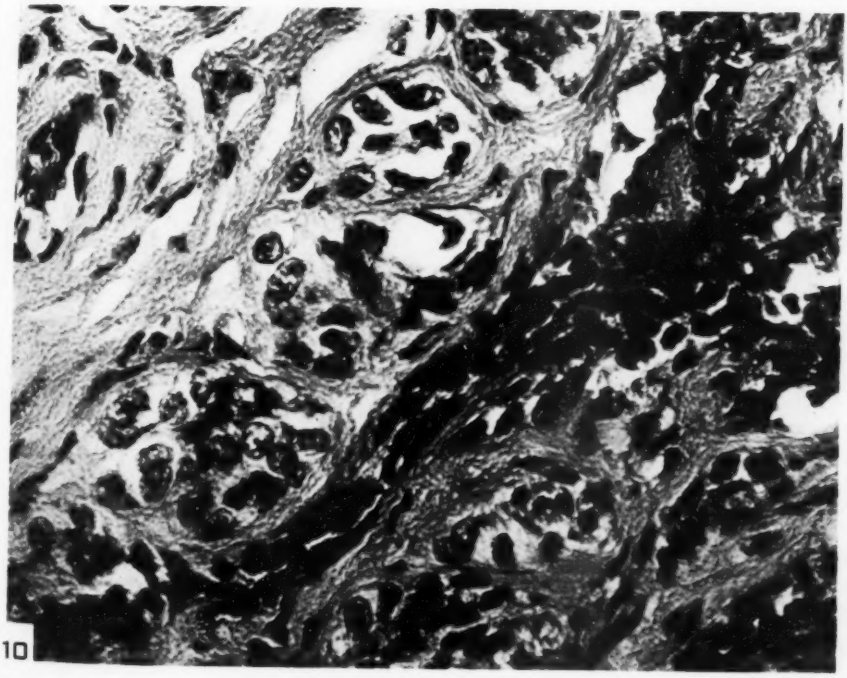

10

THE STOMACH IN PERNICIOUS ANEMIA *

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In view of the recent evidence [1] suggesting, contrary to previous reports, that in man the site of production of "intrinsic factor" is the fundus and body of the stomach, it is pertinent to reconsider the gastric changes in patients with pernicious anemia. Several reports [2-5] have described acceptably the anatomical changes in the stomach in this disease, but since the number of cases adequately studied has been small, the findings in a group of six autopsied cases of pernicious anemia in which the stomach has been studied anatomically at Stanford University have been reviewed. Two of the patients in this series differ from the reported cases in that they had continuous successful liver therapy for many years.

Table I summarizes the principal significant clinical features of the cases which are here reported. The diagnosis of pernicious anemia seems reliable in each, although the two patients (cases 1 and 2) who were under successful therapy for 13 and 10 years, respectively, had no anemia at the time of death. These, however, had had characteristic episodes in which there was rapid disappearance of anemia after the institution of liver therapy, although there is no record of reticulocyte counts from either patient. In cases 3 and 4 the patients, who had had therapy but died before the anemia disappeared, had distinct increases in blood reticulocytes following liver therapy. The fifth patient (case 5) had a macrocytic hyperchromic anemia, leukopenia, absent tendon reflexes in the legs and a history of a similar anemia 3 years previously which was diagnosed pernicious anemia and which disappeared after liver administration. During the recurrence of the anemia, liver therapy was given for only 3 days and no significant reticulocyte response had occurred at the time of death. The spinal cord showed slight demyelinization in the dorsal columns, the liver contained moderate amounts of hemosiderin, and even after the 3 days of concentrated liver therapy the bone marrow obtained at autopsy was hyperplastic and contained a few cells identified as hemoglobinized megaloblasts by Dr. Harry Wyckoff of the Laboratory of Clinical Pathology. The remaining patient (case 6) was not recognized as having pernicious anemia before death and no specific therapy was administered. This diagnosis, made at autopsy, is supported by the presence of hematogenous pigmentation

* Received for publication, September 14, 1942.

TABLE I

Clinical Data

Case	Age	Sex	Duration of treatment	Lowest red blood cell count	Reticulocyte rise	Signs of spinal cord injury	Free HCl in gastric juice after histamine
				(*million*)	(% *r.b.c.*)		
1 (OD 412)	78	M	13 years	3.1*	No record	−	0
2 (9C 177)	80	M	10 years	1.8*	No record	−	0
3 (41R-106)	42	M	2 months	2.8	12	+	0
4 (1D 86)	74	F	20 days	1.2	6	+	0
5 (1D 52)	62	F	3 days†	2.6	1.3	+	No examination
6 (ODF 7)	74	F	0	1.6	0	−	No examination

* Red blood cell count normal at death.

† Three years before death a similar anemia disappeared following liver administration.

of many of the organs and a very hyperplastic bone marrow which contained cells resembling megaloblasts.

METHODS

The stomachs to be described were all obtained at autopsy within 9 hours of the time of death. Two specimens which had remained within the body for longer than 6 hours showed superficial post-mortem digestion in some areas, but even here most of the mucosa was intact. In the other four cases the autopsy was performed between 2 and 4 hours after death and the stomachs were only slightly altered by post-mortem changes. It is felt that these changes have not significantly obscured the mucosal structure in any of the cases.

The stomachs were stretched on a board before fixation so that all mucosal folds were flattened, and, after fixation for 24 to 48 hours in a 4 per cent solution of formaldehyde, strips of mucosa were dissected from the underlying muscularis along the entire length of the greater and lesser curvatures. These were rolled up like fire hose and sections were cut after the rolls were embedded in paraffin. This procedure insures sectioning of all parts of the mucosa in a plane perpendicular to the surface and prevents fallacious interpretations of the thickness of the mucosa from tangential sections. The histological descriptions to follow were derived from examination of sections stained with hematoxylin and eosin and with Giemsa's stain.

FINDINGS

The dissection was accomplished easily. There was no unusual adhesion of the mucosa to the remainder of the stomach wall in any case. An outstanding characteristic of the stomachs was the marked alteration in the mucosa of the fundus and body, which I shall refer to as the fundic zone, in contrast to the relative freedom from abnormalities in the pyloric portion. This has already been emphasized by Meulengracht.[5] There were slight alterations in the pyloric zone in several

instances; two showed unusual numbers of cells in the interstitial tissue; in three there were scattered Russell bodies; and in two (cases 4 and 5) there were single, small, protruding mucosal nodules in this region. In cases 1 and 3 the pyloric zone was considered normal throughout and, except for the presence of the polypoid nodules, the changes of the pyloric zone in the other cases were not distinguishable from those occurring in many persons of comparable ages.

In the fundic zone the changes were extensive and severe. The mucosa was only about half the thickness of that in the pyloric zone. Even in the gross specimens this difference was apparent, producing a fairly sharp distinction between the zones (Fig. 1). The abnormal thinness was not due to post-mortem changes, but was a manifestation of a completely abnormal type of mucosa in the fundic area, in which the normal specific cell types (parietal and chief cells) were absent. The mucosal glands (Fig. 2) were shorter, less numerous and more tortuous than those of the normal fundic region. The arrangement was irregular and some glands were separated from one another by loose connective tissue. The deeper lining cells were cuboidal and fairly uniform, but were faintly stained and had no distinctive morphological characteristics. Some glands had formed small cysts, but these were not numerous in any case. Scattered through the abnormal mucosa in all stomachs were easily demonstrable, and sometimes very numerous, coarse, deeply stained glands showing structural features characteristic of mucosal glands in the small intestine (Fig. 4). Some of these atypical structures occurred singly and some were in groups which had completely replaced other glands. These glands of intestinal type contained goblet cells, and Paneth cells with prominent eosinophilic cytoplasmic granules were usually prominent in the basal portions. In case 1 similar granules were also present in the cells of small glands which were tortuous and occurred in well defined but not encapsulated clusters (Fig. 5). It will be noted that this is one of the cases treated successfully for many years. However, case 2, which also received prolonged treatment, did not present this appearance. In four of the stomachs there were considerable numbers of interstitial cells resembling lymphocytes and plasma cells among the abnormal mucosal glands, but in cases 4 and 6 these were not numerous. Similarly, although Russell bodies were abundant in the fundic zone in three cases, they were practically absent in cases 1, 3 and 4. None of the findings varied in consistent relationship to the known duration of the disease or of the therapy. The loss of specific cell types in the mucosa of the fundic zone was complete in all except case 1, in which a few small groups of atypical glands containing a few cells resembling parietal cells were present in the sections taken

from the upper portion of the lesser curvature. No chief cells were seen and no parietal cells were found in any other portion of this stomach.

The stomach from one case of nontropical sprue was examined. This patient had been studied carefully at Lane Hospital in 1928 when he had had a severe anemia, diarrhea and normal gastric acidity. The papillae of the tongue were moderately atrophic but at no time were there any neurological changes. When liver therapy was instituted there was an increase in blood reticulocytes to 16.3 per cent of the total number of erythrocytes. This was followed by a complete remission of the disease, which did not return during 2 years of observation while the patient continued to eat about one-half pound of liver three times a week. After December, 1930, he disappeared for 10 years and was next seen in an almost moribund state with a hemoglobin determination of 12 per cent and a red cell count of 600,000. In spite of attempted therapy he died after 26 hours. Many organs showed hemosiderin deposits which were particularly prominent in the liver where there was extensive diffuse scattering of fine granules of iron-containing pigment, not only in the Kupffer cells but also very prominently in the hepatic cells. In the bone marrow were accumulated large numbers of cells morphologically like the megaloblasts of pernicious anemia.

The stomach, removed 3 hours after death, showed none of the changes which characterized the stomachs of the above-described cases of pernicious anemia (Fig. 6). This is in accord with published reports of the demonstration of normal amounts of free acid in the gastric juice from patients with sprue, but does not support the view (Olleros[6] and others) that some degree of "gastritis" is present in this disease.*

Discussion

Some doubt has been expressed[8-11] whether all cases of pernicious anemia have characteristic gastric lesions, and there is little question that diseases such as sprue and infestation with *Diphyllobothrium latum* may lead to the development of a similar anemia without evidence of gastric disease. However, the gastric changes in cases of pernicious anemia, such as those here reported, are sufficiently alike and characteristic so that they may be considered as a group. Changes of so-called "chronic gastritis" are frequent in stomachs from patients who have not had pernicious anemia (Konjetzny[12] and others), but although these have some qualitative resemblance to the lesions in the

* Studies of pepsin secretion in this patient showed unusually low values which have been reported in case Ga in a series studied by Polland and Bloomfield.[7] The gastric mucosa at autopsy contained abundant chief cells, but they were less intensely stained than usual and the staining reaction was irregular.

cases of pernicious anemia, the extent of the changes and the distribution are different. Post-mortem study in this laboratory of 175 stomachs from routine autopsies by the method outlined above has shown frequent abnormalities of the mucosa of the pyloric zone, but in only four instances were changes in the fundic zone sufficiently pronounced to suggest the lesion in pernicious anemia. Even in these, scattered specific cells were present, distinguishing the stomachs from those of the cases of pernicious anemia. Usually in the absence of pernicious anemia, gastric mucosal changes are limited to the pyloric zone, or are most pronounced in this region. The lesion in pernicious anemia, therefore, may be regarded as probably different from the mucosal change which occurs in many stomachs with advancing age or with diseases other than pernicious anemia.

The cause of the stomach disorder in pernicious anemia cannot yet be pointed out. Evidence for an hereditary influence is convincing,[13] but this might lie in a predisposition of the mucosa to injury rather than in a congenital malformation. No instance of this type of gastric lesion in an embryo or child has been reported, suggesting that the change in cases of pernicious anemia is an acquired one.

It is unlikely that the lesion is a result of anemia, since achlorhydria has been observed to precede the anemia, sometimes by a period of years, and reports of return of free hydrochloric acid to the stomach after cure of the anemia are rare. The persistence of a practically normal gastric mucosa in some cases of sprue, such as the instance here reported, in spite of the presence of severe pernicious anemia, suggests not only that the anemia itself is not a significant etiological factor, but also that the factors directly causing the anemia do not produce the gastric lesions. The lack of relationship between the amount of treatment and the appearance of the mucosa in the cases here reported does not support the view[14, 15] that treatment causes disappearance of the "gastritis." The changes sometimes observed by gastroscopists during treatment of pernicious anemia might be due to growth of replacement epithelium, but the histological and functional evidence indicates that a return to a normal mucosal type does not occur.

Inflammation may be part of the process in the stomach, as cellular infiltration of the mucosa is present in some cases, but inflammation alone does not explain satisfactorily all of the observed changes. No significant relationship between the degree of cellular infiltration and the duration of the disease has been noted in the cases presented here, and there was no extensive fibrosis of the mucosa which in all parts could be dissected free from the underlying tissues very easily, in contrast to the firmly adherent mucosa in the zone of inflammation which

borders chronic ulcers. The localization of the process to the fundic portion of the stomach is not readily explained by the concept that the process is primarily inflammatory.

The irregularity of the gastric mucosa in the altered areas and the presence of atypical gland types suggest that some epithelial proliferation had occurred. The frequent presence of a few glands of intestinal type in the gastric mucosa of many people, particularly in older age groups and in association with other mucosal changes, indicates that this type of growth occurs readily in the stomach, perhaps accompanying a number of different types of disease. The frequency of this phenomenon in cases of ulcer and carcinoma of the stomach has been repeatedly emphasized.[12, 16, 17]

A possible cause of the gastric mucosal changes in cases of pernicious anemia might be some sort of selective massive destruction of the parietal and chief cells with relatively slight injury of the less differentiated cells. If such an injury were followed by limited repair associated with mild lymphoid cell infiltration, changes like those in the stomachs of patients with pernicious anemia might be produced. The appearance in the cases here described is reminiscent of the selective injury to specific epithelial cells in massive toxic necrosis of the liver. If such an episode is survived, hepatic cells may be completely removed from large portions of the liver while less differentiated cells forming bile ducts remain and proliferate. There may be relatively little newly formed fibrous tissue or evidence of inflammation. A comparable process in the gastric mucosa should be expected to affect principally the most differentiated elements—the parietal and chief cells—leaving the pyloric zone relatively unaffected. Such damage might appear only in unusually susceptible individuals in the same way that liver necrosis occurs only occasionally after cinchophen administration, or that bone marrow injury develops after chemotherapy only in a few presumably hypersensitive individuals.

Summary

In six cases of pernicious anemia the stomach showed almost complete replacement of the normal mucosal glands of the fundic type by abnormal, less differentiated glands. The pyloric zone was only slightly altered. No relationship could be found between the appearance of the stomach and the duration of the disease or of the treatment. The stomach from a well-studied case of long-standing sprue with fatal macrocytic anemia showed no comparable changes. The gastric lesions in the cases of pernicious anemia are different from those accompanying other diseases and it is suggested that they may represent a specific

change, perhaps the result of massive destruction of the highly differentiated parietal and chief cells.

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[*Illustrations follow*]

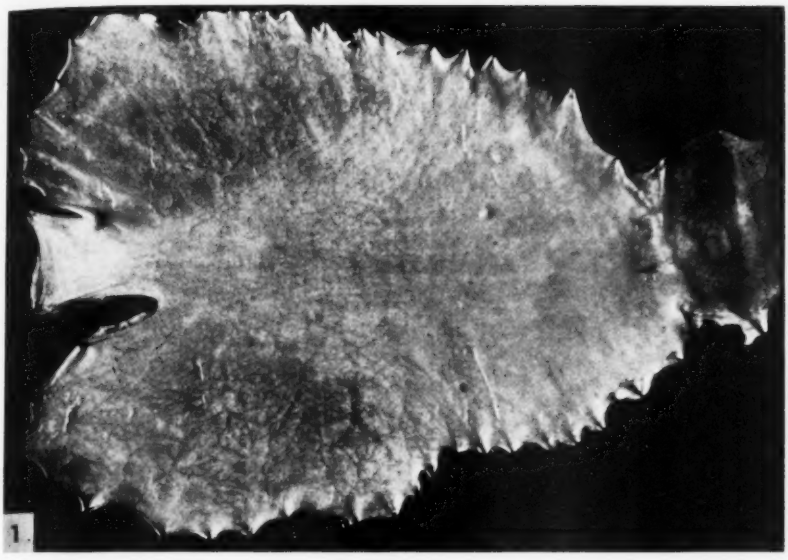
DESCRIPTION OF PLATES

PLATE 53

FIG. 1. Stomach from case 5, opened along the greater curvature, showing a very thin fundic zone with easily visible submucosal vessels, contrasted with the pyloric zone of normal thickness except for a single, small polypoid nodule. One-third natural size.

FIG. 2. Section of the fundic portion of the gastric mucosa of case 4 showing abnormal architecture with irregularly arranged, small, tortuous glands entirely devoid of parietal and chief cells. Hematoxylin and eosin stain. × 92.

FIG. 3. Section of the normal pyloric portion of the gastric mucosa from case 4. Hematoxylin and eosin stain. × 92.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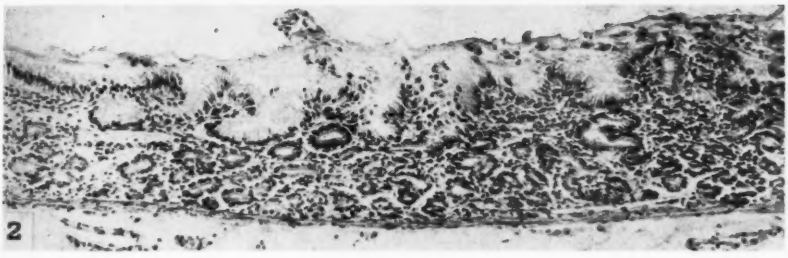
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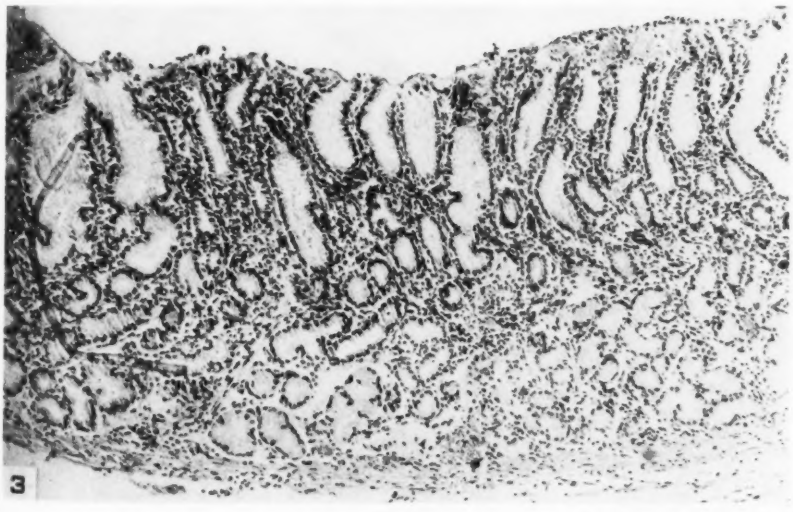
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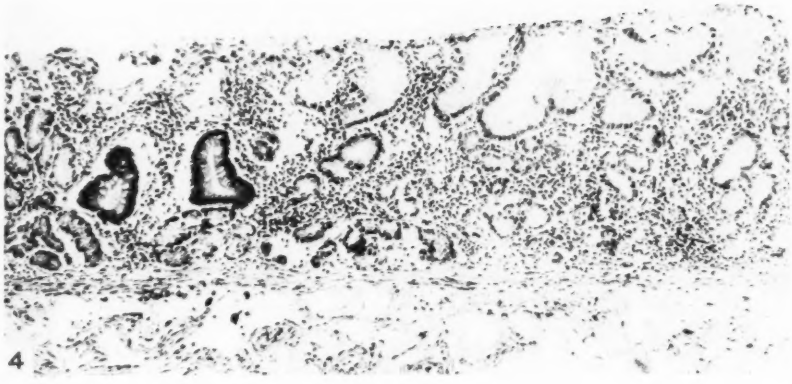
PLATE 54

FIG. 4. Section of the fundic portion of the gastric mucosa of case 1 showing interstitial cells in moderate numbers among the abnormal glands, and two darkly stained glands of intestinal type. Hematoxylin and eosin stain. × 92.

FIG. 5. Section of the fundic portion of the gastric mucosa from case 1 showing great irregularity in structure, with a localized collection of atypical small glands. Hematoxylin and eosin stain. × 92.

FIG. 6. Section of the normal fundic portion of the gastric mucosa from a case of sprue with fatal macrocytic anemia. Hematoxylin and eosin stain. × 92.



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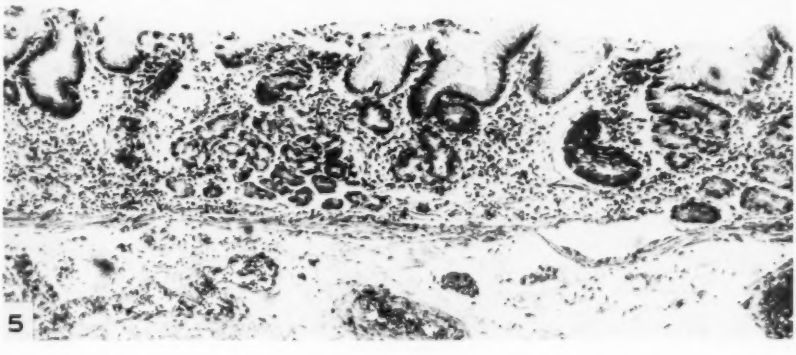

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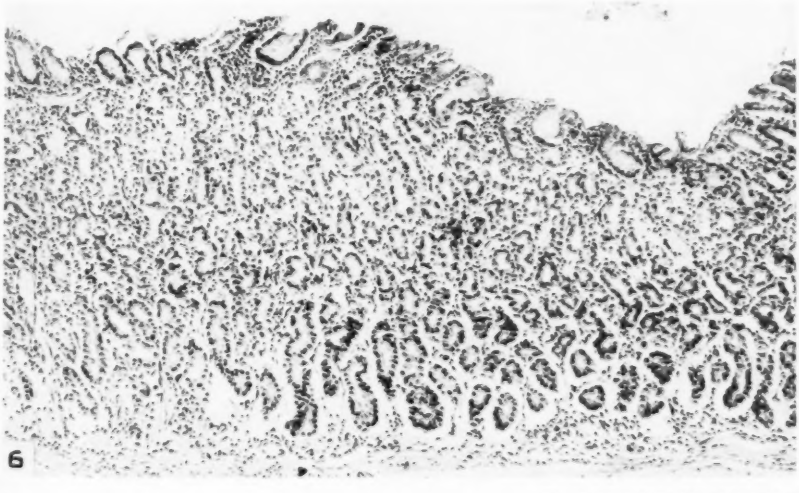

6

THE DEVELOPMENT OF THE LARVAE OF TRICHINELLA SPIRALIS IN ROLLER TUBE TISSUE CULTURES *

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The desirability of culturing the helminth parasites of vertebrates *in vitro* has been repeatedly emphasized. Hoeppli, Feng and Chu (1938) reviewed this problem and concluded that while various adult worms could be kept alive in sterile artificial media for long periods of time, in no case had marked growth and tissue differentiation of larvae been obtained in cultures. Since that time several workers have reported progress, particularly those working with strigeid metacercariae (Ferguson, 1940). However, to date, although Glaser and Stoll (1938) were able to culture the free-living stages of *Haemonchus contortus* and Ackert, Todd and Tanner (1938) were able to obtain an increase in the size of immature *Ascaridia lineata* which were obtained from the intestines of chickens, it has not been possible to obtain sexual differentiation of the parasitic stages of the nematodes of vertebrates. McCoy (1936) attempted to grow trichinella larvae in abnormal environments, pointing out that due to lack of host specificity, rapid growth to maturity, and the ease with which sterile larvae could be obtained, this parasite should prove to be a favorable species for such study. No development occurred in McCoy's Maitland tissue cultures, or in the lumina of nonpregnant rat uteri, or in the amniotic sacs of dead rat embryos, but a small number of larvae developed to sexual maturity in living chick embryos and in the amniotic sacs of living rat embryos.

The present paper reports experiments in which an attempt was made to obtain development of trichinella larvae in roller tube tissue cultures. As far as can be determined, the only previous application of this particular technic to the culture of helminth parasites is that of Mendelsohn (1935) who kept larvae of *Taenia crassicollis* alive for 35 days, but was unable to obtain significant developmental changes.

MATERIAL AND METHODS

Isolation of Larvae

Trichinella larvae were obtained by peptic digestion of stock mice which had been infected from 5 weeks to 6 months before use. The carcasses were skinned, the feet and head cut off and the viscera removed.

* Study initiated with the aid of the George Cheyne Shattuck Memorial Fellowship, Harvard Medical School.

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Gross fecal contamination was avoided by tying off the esophagus and rectum before removal. The carcasses were then washed in cold running water and passed through a meat grinder. In early experiments the trichinous material was digested in battery jars and the larvae concentrated by sedimentation. Later, a modified Baermann apparatus similar to that described by Hobmaier and Meyer (1937) was used. This proved to be a simple technic for obtaining viable larvae that were relatively free from contamination. The meat grinder, Baermann apparatus, glassware and instruments were sterilized by autoclaving before each use. The digestion mixture routinely consisted of 4 gm. of pepsin, 5 gm. of sodium chloride and 9 cc. of hydrochloric acid (sp. gr., 1.19) in a liter of tap water. Digestion was carried out at 37° C. for a period of 6 to 8 hours; longer periods of digestion were found to decrease the number of viable larvae.

Sterilization of Larvae

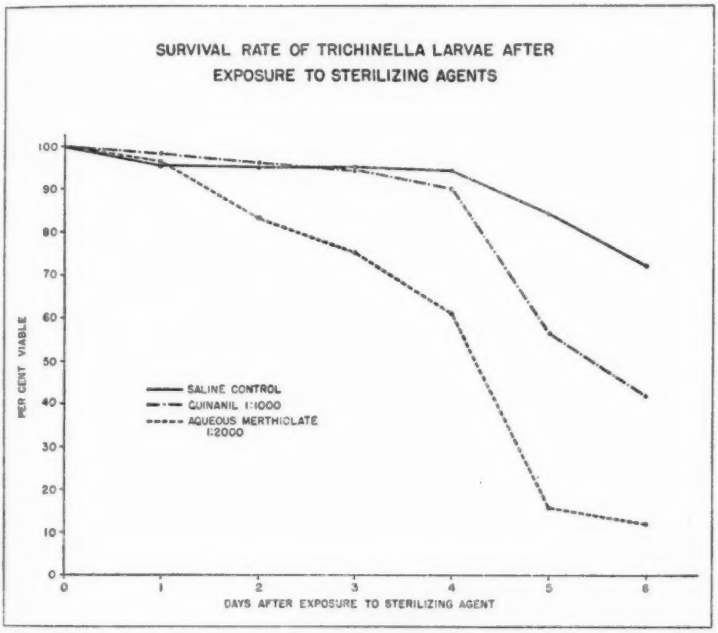
Several methods of sterilizing larvae were used. Routinely, the procedures of "sterilization" and washing were carried out in 50 cc. centrifuge tubes. The larvae were introduced into 25 cc. of the sterilizing agent with a sterile Pasteur pipette, agitated for 2 minutes and then allowed to settle for 3 minutes. Following this they were washed by transfer through five tubes, each of which contained 25 cc. of normal saline solution; sterile pipettes were used for each transfer and the larvae were left 5 minutes in each tube.

In the early studies, a 1:2000 solution of aqueous merthiolate, buffered with 0.07 per cent borax, as recommended by McCoy (1936), was used as a sterilizing agent. Although McCoy demonstrated that a small proportion of larvae so treated possessed the ability to develop to maturity, as the present study progressed and complete development in tissue cultures was not obtained, it seemed advisable to determine whether the sterilizing agents employed had any latent deleterious effect. At the same time the possibility of using other methods of sterilization was investigated. Glaser and Stoll (1940) used sodium hypochlorite solutions for sterilizing and exsheathing nematode larvae. Boxhall, Happold and Lloyd (1934) found quinanil * to be an effective bactericidal agent in the isolation of a flagellate from fly intestines, and therefore its use was suggested.

Simple *in vitro* experiments were set up to determine the longevity of larvae after sterilization. A typical protocol follows:

* Quinanil is the trade name for 2(p-dimethyl-amino-anil) 6(methyl-quinolene methochloride) and is produced by The British Drug Houses, Ltd., London.

Sterility Experiment No. 6. Following digestion, 100 active larvae were picked up with a mouth pipette, using a dissecting microscope, and placed in each of three Wassermann tubes containing, respectively, 5 cc. of normal saline solution, 5 cc. of a 1:2000 solution of aqueous merthiolate buffered with 0.07 per cent borax and 5 cc. of a 1:1000 normal saline solution of quinanil. The larvae were left in the sterilizing solutions for 5 minutes. They were then washed by transferring



Text-Figure 1. Graphic tabulation of the survival rate of the larvae of *Trichinella spiralis* after exposure to 1:1000 quinanil and 1:2000 aqueous merthiolate solutions, compared to a control group treated with normal saline solution.

each lot of larvae with a sterile Pasteur pipette through five tubes, each of which contained 5 cc. of normal saline solution; the larvae were left 5 minutes in each tube. Following washing, each lot was placed in 5 cc. of normal saline solution in a Wassermann tube and was incubated at 37° C. Each day the larvae were transferred to a Syracuse watch glass, and the live larvae counted and returned to the tubes of saline solution. Unless motion was seen, larvae that were uncoiled or showed definite degenerative changes were arbitrarily assumed to be dead. The results of this experiment are presented in Text-Figure 1 and show that

merthiolate when used as a sterilizing agent in this manner has a definite toxic effect which first becomes apparent 48 hours after the larvae are exposed. Quinanil has a similar but less marked effect. In similar experiments, a 5-minute exposure to a 1:500 dilution of sodium hypochlorite solution U.S.P. proved to be more toxic than merthiolate.

In an effort to avoid the toxic effect of chemical sterilizing agents an attempt was made to free the larvae of contaminating bacteria mechanically. By using the precautions outlined above in the section on isolation of larvae, and then by washing them for 5 minutes in each of six tubes containing 25 cc. of normal saline solution, a nematode suspension was obtained that was bacteriologically sterile when cultured aerobically and anaerobically, and which proved to be satisfactory as an inoculum for roller tube tissue cultures.

Attempts to Obtain Development in Roller Tube Tissue Cultures

Basic Technic. The basic technic employed was similar to that used recently for the culture of vaccinia virus (Feller, Enders and Weller, 1940). Reference should be made to this paper for details of the method. In brief, the cultures were prepared by planting fragments of minced 8- to 10-day-old chick embryo tissue in a chicken plasma clot distributed evenly over the wall of a 20 by 150 mm. pyrex test tube. Nutrient fluid consisting of 1.6 cc. of a mixture composed of Simms' solution, 7 parts, chicken embryonic extract, 2 parts, and chicken serum, 1 part, was added and the tube was then sealed with a one-holed rubber stopper fitted with a short piece of pyrex tubing, which in turn was closed with a rubber vaccine bottle cap. The cultures were placed horizontally in a rotating device which revolved 8 to 10 times every hour and were kept in an incubator at 37° C. Each day, after observations had been made, the nutrient fluid was removed through the small pyrex tube by means of a Pasteur pipette and 20 cc. of air which had been drawn through sterile cotton with a syringe was then introduced. Fresh nutrient fluid was added, the tube was sealed and returned to the incubator.

From 50 to 300 sterile larvae were introduced into each roller tube from 6 to 24 hours after the culture was assembled, with the shorter period of time giving the better results. Observations on the cultures were made with a low-power objective. Each time nutrient fluid was removed, the relatively few nemas suspended in the fluid were studied alive and then were fixed in a warm 4 per cent solution of formaldehyde. For the purposes of photomicrography, a few cultures were set up using roller bottles as described by Shaw, Kingsland and Brues (1940). These proved to be very satisfactory.

Results Obtained with the Basic Technic

Using the technic described above, partial development of the trichinella larvae was obtained. Although the nemas showed a decrease rather than an increase in size, and progressively died off, a small percentage molted twice and developed to the point of sexual differentiation.

Nineteen cultures were set up at various times using the basic technic. While there was some individual variation in the tubes, in general the results can be summarized as follows. Within 30 minutes after introduction of the larvae into the cultures, they showed vigorous activity, coiling and uncoiling rapidly, and then moving with a serpentine motion through the tissue. As migration began, the anterior tip vibrated rapidly, giving the impression that the nema was feeding. Although larvae molted for the first time as early as 16 hours after inoculation, the first ecdysis usually occurred between 24 and 36 hours after the culture was set up. Prior to molting there was a decrease in length, with retraction of the larva away from the old cuticular sheath both posteriorly and anteriorly. Coincidentally, a decrease in motility occurred, with movement being limited to a "to and fro" motion within the sheath and a fine vibratory motion of the anterior end. Molting was a slow process; one nema, which when first observed was half way out of its sheath, required 30 minutes to complete the procedure, which was accomplished by a slow backward and forward movement, accompanied by a lashing movement of the anterior free portion (Fig. 1). Upon completion of the molting process, the larvae were again extremely active and appeared to be feeding among the growing cells. The newly-escaped "second stage" larvae could be distinguished from those that had not molted by being shorter and thicker. They showed no sexual differentiation (Fig. 3).

From 10 to 20 per cent of the total number of larvae completed the first molt. Others failed to complete the first molt but began to show retraction in preparation for a second molt while still within the first sheath (Fig. 2). While many of the larvae that had molted once continued to develop and to show changes that were interpreted as being in preparation for further molts, only twice was the second molt observed; this occurred 48 hours after inoculation. These nemas showed a further decrease in size and the cast-off cuticula was smaller and more delicate than that shed during the first ecdysis. No sexual differentiation could be seen in this stage.

Further development was observed in larvae that failed to molt completely, but instead carried out an "incomplete molt" so that one sheath lay within the other. Retraction from a third cuticular sheath was first

seen at 38 hours and occurred frequently by the 50th hour of cultivation. Larvae that had retracted from the third sheath showed sexual differentiation with development of the vulva, ovary and uterus in the female and the appearance of anal papillae in the male (Figs. 4 and 8). By the 65th hour of cultivation such larvae had begun to show degenerative changes with beginning loss of internal structure; however, coincidentally there was a retraction from a fourth sheath (Fig. 6). In the male, the fourth sheath showed posteriorly a "cast" of the anal papillae (Figs. 5 and 7).

Only a small proportion of the larvae in each tube showed the developmental changes described above. Usually 80 per cent were alive at 24 hours, and about 60 per cent at 48 hours. By the end of the third day, degenerative changes began to appear and development ceased during the fourth or fifth day after inoculation, although a few degenerating larvae remained alive for as long as 9 days. During the period of development there was a decrease rather than an increase in size. Fifty larvae, killed in a warm 4 per cent solution of formaldehyde immediately after digestion, averaged 899 μ in length (extremes 805 to 1220 μ). Ten males which had molted once, lying in two cuticular sheaths, and which showed well developed anal papillae, were killed in a warm 4 per cent solution of formaldehyde after 54 hours of incubation; they averaged 806 μ in length (extremes 670 to 950 μ). Ten comparable females, each showing a well developed vulva, averaged 814 μ in length (extremes 700 to 925 μ). The presence of the nemas in the cultures did not affect tissue growth. As in the virus experiments, the relatively large amounts of tissue used grew rapidly, with an accompanying fall in the pH from an initial value of about 7.8 to 7.0 or below in 24 hours.

Attempts to Obtain Further Development by Modification of the Basic Technic

Numerous experiments were carried out in an attempt to improve the technic described above. In each experiment one control roller tube was set up using the basic technic. Of the many combinations tried, not one proved to be more satisfactory than did the technic outlined above.

An attempt was made to determine if any of the components of the nutrient media had a deleterious effect upon the larvae. Sterile larvae were placed in Wassermann tubes containing 10 per cent chicken serum in normal saline, 20 per cent embryonic extract in normal saline, Simms' solution, and normal saline solution. After incubation for 72 hours at 37° C., it was found that there was no significant difference between

the number of viable larvae in the control saline tube and in the embryonic extract and Simms' solution tubes; however, in the 10 per cent chicken serum tube, there were only one-fourth as many viable larvae. Therefore, roller tubes were set up using a nutrient fluid composed of two parts of embryonic extract and eight parts of Simms' solution; both larval development and tissue growth were poorer than in the control tube. Roller tubes were then set up using a nutrient fluid in which sheep serum was substituted for the chicken serum; excellent tissue growth but poor development of the nemas resulted. Mammalian embryonic tissue was substituted for the chick tissue by setting up roller tubes using rat embryos of approximately 18 days' gestation; this modification resulted in no significant change in the amount of development. Similar findings were obtained in an experiment planned to determine the effect of using various types of tissue in which 12-day-old chick embryos were dissected and separate roller tubes planted with liver tissue, intestinal tissue, and brain tissue. The possibility that the presence of an abrasive substance might assist in molting was studied by distributing sterile sand throughout the plasma coagulum in one set of tubes; no change was noted in the development of the larvae.

The behavior of the nemas suggested that some essential growth factor or factors might be lacking. A yeast extract was made using the method of Ferguson (1940); this was added to the nutrient fluid in various concentrations up to 10 per cent, either alone or in conjunction with added liver extracts. Liver extracts, which were used in concentrations up to 5 per cent, were prepared from Eli Lilly extract no. 343 by the method of Glaser and Coria (1933), and also from a crude aqueous liver concentrate * (1 cc. of concentrate was the equivalent of 0.03 lbs. of liver). The latter was employed by making a 1:1250 dilution in normal saline and sterilizing the solution by passage through a Seitz filter. Other cultures received nutrient fluid which, in addition to the usual components, contained 1 μg. of ascorbic acid and 4 μg. of glutathione per cc.; another set was given fluid containing 5 μg. of thiamin hydrochloride and 1 μg. of riboflavin per cc. In one group of tissue cultures the effect of adding split protein products was tried; to the standard nutrient media, an equal amount of 5 per cent aqueous bacto-tryptose, bacto-tryptone, or bacto-proteose peptone no. 3 † was added. In another set, 2.9 mg. of casein hydrolysate * was added to each 10 cc. of nutrient medium. All of the fluid media listed above gave fair to good tissue growth but did not significantly affect the development of the nemas. The addition of fresh human bile in concentrations of from 0.05 to 0.5 per cent and of sodium thioglycolate in concentra-

* Supplied through the courtesy of Lederle Laboratories, Inc., New York, N. Y.
† Produced by Difco Laboratories, Inc., Detroit, Michigan.

tions of from 0.001 to 0.005 per cent had a definite toxic effect upon the larvae.

Discussion

Although numerous modifications of the basic technic failed to provide an environment that permitted complete development of trichinella larvae, it would appear from the results of the present study that the roller tube technic deserves further investigation, and may with only slight modifications prove to be a useful tool in the study of the helminthic parasites.

While no detailed morphological studies were made, the present findings are of interest in view of the uncertainty that exists regarding the number of molts that trichinella larvae undergo while developing in the intestine. Kreis (1937) recovered the developing larvae from infected rats at regular intervals and concluded that the female passed through four molts and the male through three molts. He noted no sexual differentiation until after the second molt, which occurred between the 12th and 16th hours of intestinal life. In the present study, sexual differentiation was first seen coincidentally with retraction from the third sheath. While the cultural results agree with those of Kreis as to the total number of molts in the female, in the present study males also were seen which had molted once and in addition were enclosed in three cuticular sheaths. This finding suggests that the male has four molts, although it is possible that one or more of the cuticular sheaths represent a response of the larva to the abnormal environment or else is a degenerative change. Chandler, Alicata and Chitwood (1941) felt that Kreis' evidence was not convincing and stated that "according to recent investigations one molt was obtained after ingestion and the cuticle of the resultant nema passed uninterrupted over the vulva, indicating that at least one more molt would be necessary before maturity." Inasmuch as in the present study the vulva was not seen until about the time of the third molt, this statement also conflicts with the observations reported above.

Summary

In roller tube tissue cultures trichinella larvae developed to the stage of sexual differentiation. While the nemas decreased rather than increased in size, a few larvae completed two molts. A larger number of larvae completed one molt, and then progressed through three additional "incomplete molts," so that nemas were seen lying within three distinct cuticular sheaths. Anal papillae in the male and the vulva in the female became prominent after the third "incomplete molt." These findings suggest, but do not prove, that both male and female trichinella larvae molt four times during the intestinal phase of their life cycle.

Agents previously used for sterilizing nemas were found to have a toxic effect on trichinella larvae and therefore a simple washing technic was developed which yielded bacteriologically sterile larvae that were suitable for introduction into tissue cultures.

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[*Illustrations follow*]

DESCRIPTION OF PLATES

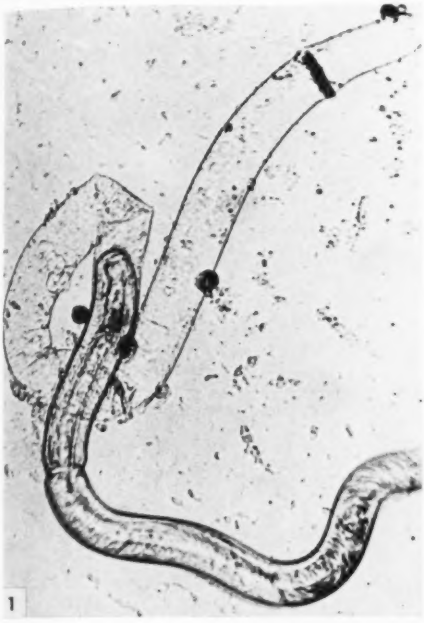
PLATE 55

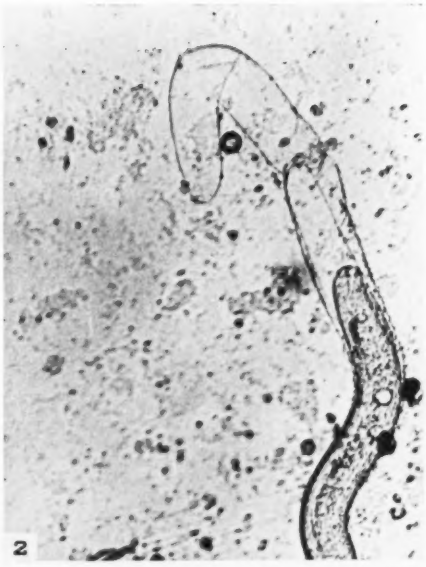
FIG. 1. Live larva (*Trichinella spiralis*) molting for the first time. The free portion was moving rapidly. Photographed in roller bottle tissue culture after 26 hours' incubation.

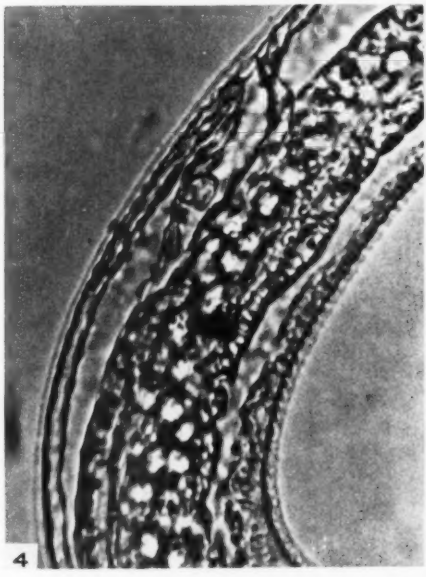
FIG. 2. Live larva in roller bottle tissue culture after 38 hours' incubation showing an "incomplete" first molt and retraction from a second cuticular sheath in preparation for a second molt.

FIG. 3. Live larva in roller bottle tissue culture shortly after completing the first molt. Taken after 28 hours' incubation. The anterior tip was vibrating rapidly.

FIG. 4. View of vulvar region of female nema shown in Figure 8. Heat-killed after 48 hours' incubation.







Weller Development of *Trichinella spiralis*

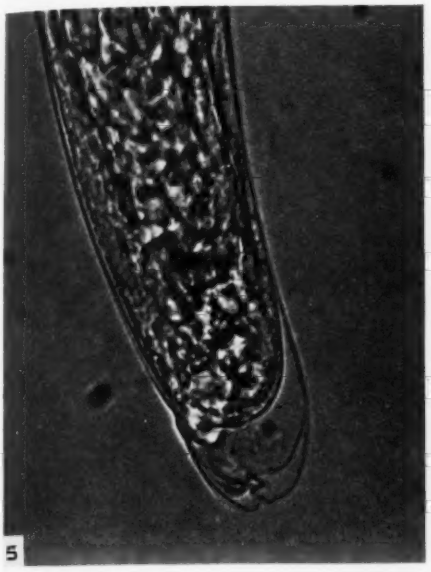
PLATE 56

FIG. 5. Posterior end of male with well developed anal papillae. This nema probably had molted twice, and lies within a smooth third cuticular sheath, and a fourth sheath that shows a cast of the anal papillae. Heat-killed after 58 hours' incubation.

FIG. 6. Posterior end of female that had molted once, showing retraction from three additional cuticular sheaths. Heat-killed after 70 hours' incubation.

FIG. 7. Low-power view of male shown in Figure 5. Shows maximum development obtained. Heat-killed after 58 hours' incubation.

FIG. 8. Low-power view of female pictured in Figure 4, showing maximum development obtained. This nema had molted once, and was lying within two additional cuticular sheaths. Heat-killed after 48 hours' incubation.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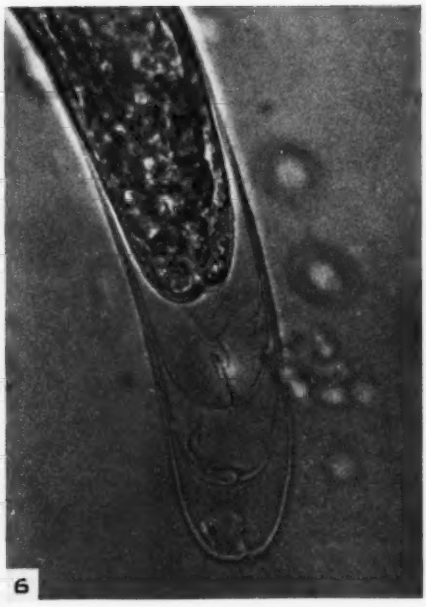
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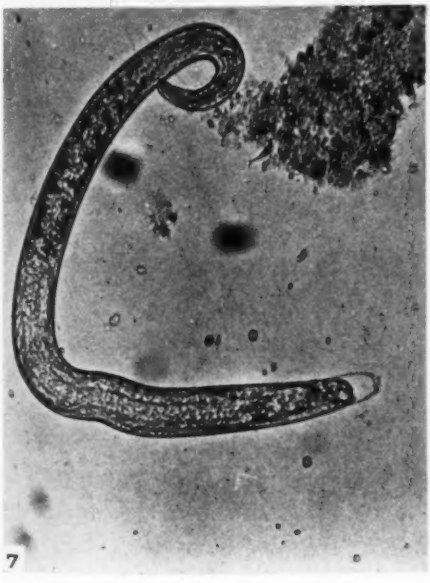
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8

Weller Development of *Trichinella spiralis*

EFFECTS OF INFRARED IRRADIATION ON THE TISSUES OF THE RABBIT *

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Many data have accumulated on the effects of roentgen irradiation on both normal and pathological tissues, yet the exact method by which the effects of these rays are produced is not thoroughly understood. Ellinger [1] has recently compiled much of the literature on the mode of action of irradiation on cells. Few studies, however, have been made on the pathological changes that follow ultraviolet irradiation and still fewer on the effects produced by infrared.

All types of irradiation may produce injury to cells, according to the photochemical theory. Various factors, of course, influence these changes. The type of cell, the degree of absorption and the intensity of the irradiation are important. Roentgen rays are very penetrating and carry large amounts of energy. Ultraviolet radiations are characterized by their less penetrating powers; however, they initiate chemical changes in a large number of biologically important substances.[1] The ultimate effects of infrared irradiation may be the same as roentgen and ultraviolet irradiation.

Recently experimental studies on the effect of roentgen irradiation on capillary permeability and inflammation, and of ultraviolet irradiation on the localization and concentration of antibodies in the skin of the rabbit have been made.[2, 3] Infrared irradiation also was used to determine its effect on these processes. Marked pathological changes occurred in the skin and viscera of the rabbits treated with infrared rays. This paper is a report of these effects.

Methods and Materials

Adult rabbits were used. The hair was shaved from the sides and abdomen 24 hours or longer before the experiments were begun. Infrared irradiation was obtained from a lamp † placed 10 inches above the area of skin to be irradiated. The electric current used in this lamp was 110 volt, 60 cycle A.C.; 220 watts. The energy distribution graph with the spectral distribution of the output of this lamp is shown in Text-Figure 1.

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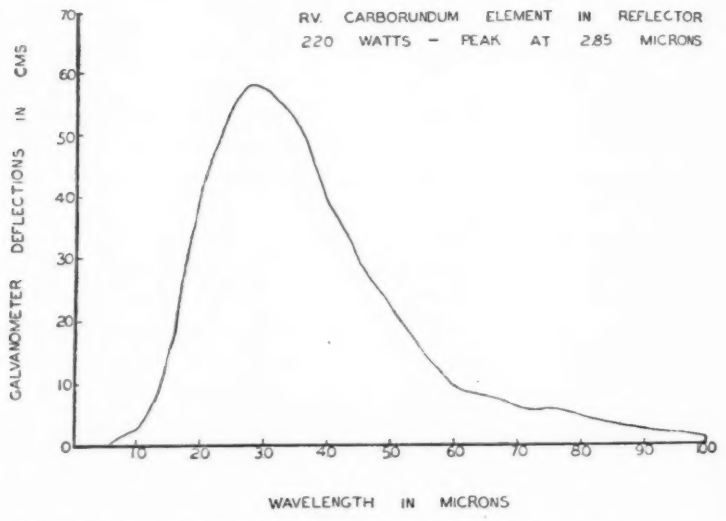
† The lamp is a Zoalite, type Z-70, supplied by the Burdick Corporation, Milton, Wisconsin. The data for Text-Figure 1 were also furnished to us by that company. We acknowledge our appreciation for this co-operation.

A cloth towel with a hole 3.5 cm. in diameter was placed over the shaved skin of some of the animals. Some of the rabbits were anesthetized with pentobarbital when the exposure was made for a long period. The length of the exposure of the skin of these animals to infrared varied from 1 to 5 minutes. The longer intervals were used to produce lesions in the viscera.

Ten cc. of a 0.2 per cent solution of trypan blue was injected intravenously immediately following irradiation. The rabbits were killed at intervals varying from immediately after injection to 144 hours. Autopsies were performed at once to determine the areas in which the



Text-Figure 1. The energy distribution graph with the spectral distribution of the output of the lamp used in these experiments.

trypan blue had localized and concentrated. Sections were removed and fixed immediately in a 4 per cent solution of formaldehyde. Paraffin sections were prepared and stained with hematoxylin and eosin.

Effect of Infrared Irradiation on the Rabbit as Observed by the Intravenous Injection of Trypan Blue

Infrared light, when applied to the skin of the rabbit, produced almost instantaneous blanching and then, very quickly, hyperemia. Edema subsequently developed. Trypan blue, when given intravenously immediately following the irradiation, localized and concentrated throughout this hyperemic area. When the amount of irradiation was markedly

increased, the tissue directly beneath the arc of the lamp remained pale yellow in color, and a zone at the periphery became hyperemic and edematous. Trypan blue was localized and concentrated only in the zone about the periphery of the pale yellow area. Histological studies made subsequently indicated that the cells were completely destroyed in the center of this area and only injured about the periphery. This observation indicates that trypan blue will be localized and concentrated only in areas where the cells are injured and not completely destroyed.

When infrared irradiation was applied to the skin for a period of 5 minutes and trypan blue was given intravenously immediately thereafter, the dye localized and concentrated in the abdominal muscle and in the portion of intestine just beneath the irradiated area of skin. It was apparent that infrared rays, as used in these experiments, penetrated the abdominal wall of the rabbit and produced changes in the permeability of the cells in the intestines similar to the changes that occurred in the skin.

Histological Lesions Observed in the Rabbit Following Infrared Irradiation

Skin. The epithelial cells showed injury of various types. These were influenced by the length of the exposure. The subcutaneous tissue became edematous and its cells pyknotic. Many cells resembling plasma cells infiltrated the corium. Polymorphonuclear leukocytes sometimes infiltrated the corium immediately beneath the squamous epithelium. The endothelial cells of the small blood vessels became swollen and pyknotic. Thrombi in the smaller blood vessels were rarely seen except in those rabbits given very large amounts of the irradiation.

Muscle. The muscle in the abdominal wall appeared to be very susceptible to the effects of infrared irradiation. Groups of muscle cells frequently became swollen and lost their striations within a period of 30 minutes following irradiation. Many of these muscle cells were necrotic and were fragmented within an hour. Polymorphonuclear leukocytes frequently infiltrated the tissue about these degenerating muscular fibers.

Gastrointestinal Tract. Ulcers were found to occur in any portion of the intestinal tract following exposure of the abdomen to infrared irradiation for a period of 3 to 5 minutes. It was necessary to concentrate the irradiation in the area of the stomach to produce ulcerative lesions in that organ. Likewise, it was necessary to place the light over the skin in the area of the small intestine and colon to produce lesions there. The earliest lesion observed macroscopically was hemorrhage

into the mucosa. Apparently, lesions such as this subsequently developed into ulcers. The size and number of ulcers varied in different rabbits. Lesions in the stomach and the cecum are shown in Figures 1 and 2.

The epithelial cells apparently were very susceptible to the effect of infrared irradiation. These cells showed all types of degenerative changes. Karyorrhexis was very marked in some of the lesions. Figure 3 shows the accumulation of degenerated epithelial cells in the lumen of the glands in the mucosa of the colon. Similar changes occurred in the epithelial cells in the stomach and the small intestine. The entire wall of the colon became edematous in the area of the ulcers. Polymorphonuclear leukocytes infiltrated the portion of the intestine injured by irradiation.

The cells in the lymphoid tissue in the intestinal tract appeared to be much more resistant to the action of infrared irradiation than the columnar epithelial cells. The epithelial cells covering a Peyer's patch and also the cells lining the glands in the area were severely injured by infrared irradiation while the lymphocytes did not show any specific degenerative changes (Figs. 4 and 5).

Liver. Focal yellowish red areas were present in the livers of some of the rabbits. This lesion extended for only a short distance into the hepatic tissue. Always the lesion occurred at the periphery of the organ. Histologically the hepatic cells in these areas showed all stages of injury from cloudy swelling and vacuolization of the cytoplasm to karyolysis. In some of the animals, polymorphonuclear leukocytes infiltrated the area of degeneration. There was always a sharp line of demarcation between the injured and the normal cells (Fig. 13).

Spleen. Lesions seldom occurred in the spleen of this group of rabbits. Figure 10 shows several focal areas of necrosis. These were definitely localized and appeared as infarcts. Histologically the splenic tissue showed degenerative changes, and polymorphonuclear leukocytes were found in a pyogenic membrane about the periphery of the area. It is important to observe (in Fig. 12) a splenic vessel in this area, in which the lumen is occluded by a pink-staining, fibrinlike material. The wall is necrotic. The presence of this vascular lesion suggests that the degeneration in the surrounding splenic tissue results from it rather than from the direct effects of infrared irradiation on the splenic tissue, but that the lesion in the vessel was itself due to irradiation.

Kidneys and Adrenals. No pathological changes were observed in the kidneys and adrenals. This may be attributed to an insufficient quantity of infrared irradiation reaching these tissues.

Lungs. A few of the rabbits showed focal hemorrhagic areas and some edema in the lungs following irradiation of the abdomen. A more

extensive lesion occurred when the light was applied to the skin directly over the chest wall.

Heart. No definite pathological lesions were observed.

Femoral Bone Marrow. A posterior extremity of each of five rabbits was shaven. Infrared irradiation was applied to the thigh for a period of 5 minutes. Six hours later the animals were killed and the femoral bone marrow removed. Marrow was removed from a corresponding portion of the opposite leg. There were no pathological changes observed in the sections of the marrow from either leg.

Cranial Bone Marrow. A portion of the skull in the area treated with infrared irradiation was removed from some of the rabbits. The marrow here was hemorrhagic and the cells showed marked degenerative changes. The skull in the rabbit is relatively thin as compared with the shaft of the femur. It is likely that the femur is too dense for the penetration of these infrared rays.

Brain. Lesions were present in the brain of each rabbit in which infrared irradiation was used on the head. The light was applied to the area between the ears. The underlying portion of the brain was the only portion to be affected (Fig. 6). Petechiae occurred along each side of the median line. The brain cells in this area were pyknotic and showed karyorrhexis. Clear spaces were present around many of these cells. These areas were thought to represent areas of edema. The tissue was necrotic in some of the animals (Figs. 7 and 8). A leukocytic zone was present in the brain when an interval of several hours elapsed between the application of the light and the taking of the section (Fig. 9). The pathological changes in the brain usually extended for only a short distance into the cortex; however, in a few of the brains the lesions extended almost to the lateral ventricle.

Eye. The soft tissues about the eyes of rabbits receiving infrared irradiation over the skull were edematous and hemorrhagic. The epithelial cells of the cornea were frequently infiltrated with polymorphonuclear leukocytes. The cells in the retina also showed degenerative changes and leukocytes infiltrated the area. The lens frequently appeared opaque in the rabbits that lived for several hours following application of the light.

Discussion

The results of these studies indicate that a change may occur in the tissues immediately following infrared irradiation. The localization and the concentration of trypan blue following an intravenous injection indicate that these tissues vary from the normal. The subsequent histological lesions are a confirmation of this injury.

It is widely held that a histamine-like substance is liberated from the

tissues following irradiation and that the subsequent changes result from the presence of this substance.[4] It has been suggested, however, that the local changes which occur in inflammation may result from the direct action of the injurious agent on the cells rather than from the effect of a substance liberated from injured tissue.[5] The direct effect of these rays on endothelial cells in the blood vessels of the corium apparently is sufficient to produce changes in these cells that result in an increased permeability. Borak[6] has recently expressed the opinion that x-rays act on the large protein molecules to break them down to smaller ones, hence increasing the osmotic pressure and causing the flow of liquid into the cells, often reaching the point of cellular disintegration. This phenomenon occurs in both the endothelial cells and the extravascular tissue, according to Borak.

The presence of degenerative lesions in the intestinal mucosa indicates that infrared rays penetrated the abdominal wall.

It is impossible to compare the results of the effects of infrared irradiation in this study with the results of others with roentgen rays; however, it is of interest to note that Ellinger,[1] in 1941, stated that the adult brain and nervous system are relatively radioresistant; muscle is one of the least radiosensitive tissues; the kidneys are not particularly radiosensitive; the liver is decidedly radiosensitive; the intestines also are radiosensitive. It appears from the present study that the skin, muscle, gastrointestinal tract, liver and brain are very susceptible to the effects of infrared irradiation. The apparent resistance of the heart and kidney to infrared irradiation may be explained by the decreased amounts of irradiation reaching these organs.

Warren and Whipple[7] observed that the epithelial cells in the mucosa of the gastrointestinal tract were more susceptible to the effects of roentgen irradiation than the lymphoid cells in the underlying Peyer's patches. It is of interest to observe a similar relationship between the susceptibility of the epithelial and the lymphoid cells following the application of infrared irradiation to the intestines of the rabbit.

Degenerative changes in the cells of the bone marrow in the skull and the absence of lesions in the same cells in the femoral bone marrow would suggest that an insufficient amount of radiation is absorbed by the latter cells. Hofmann[8] and Heald[9] have shown that infrared radiation is transmitted through the hand and forearm of man. These observations of Hofmann and Heald, however, indicate that the rays penetrated only the soft tissues and not the osseous tissue. It would appear likely from these histological studies that infrared irradiation may produce cellular degeneration in any tissue if the concentration of radiation reaching the tissue is sufficient.

A knowledge of the potential effects of infrared irradiation is important in view of its increased clinical use. In regard to its use, Beaumont[10] has said: "It is evident to all those who have extensive opportunities for the close clinical observations of a large number of cases that there is some factor other than heat responsible in some measure for the results obtained."

Summary

The pathological changes that occur in the rabbit following the application of infrared irradiation to the skin are described. These are characterized by extensive necrosis and ulceration. Lesions are present in the skin, abdominal muscles, stomach, intestine, spleen, liver, lungs, brain, eyes and bone marrow.

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[*Illustrations follow*]

DESCRIPTION OF PLATES

PLATE 57

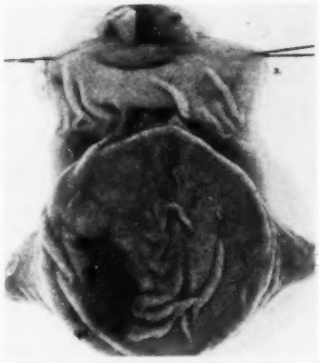
FIG. 1. Rabbit 1239. Acute ulcer in the cecum. Lesions similar to this occur also in the colon and small intestines. Usually they are multiple. Infrared irradiation was applied to the abdomen for 5 minutes at a distance of 10 inches from the skin. The cecum was removed 5 hours following irradiation.

FIG. 2. Rabbit 1234. Ulcers in the mucosa of the stomach. They are similar to those in the cecum. This rabbit was given the same irradiation as rabbit 1239, the cecum of which is shown in Figure 1, and was killed 24 hours later.

FIG. 3. Rabbit 1101. The epithelial cells lining the glands in the mucosa of the colon show extensive degenerative changes. The lumina of the individual glands are usually filled with cellular débris. This rabbit was irradiated for 4 minutes at a distance of 12 inches. Section removed 30 minutes following the irradiation. × 100.

FIGS. 4 and 5. Rabbit 1137. The epithelial cells covering a Peyer's patch and those lining the glands show an extensive degenerative change. No changes are observed in the lymphocytes. The section shown in Figure 5 was removed immediately below X in Figure 4. The skin was irradiated for 4 minutes at a distance of 12 inches. Killed 15 minutes following irradiation. Figure 4, × 36; Figure 5, × 375.

1



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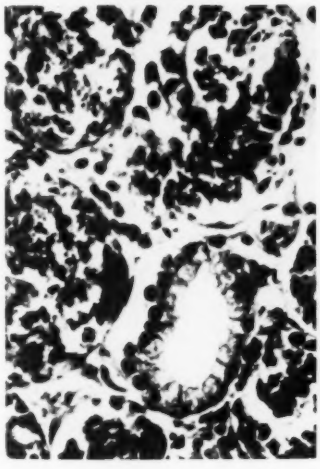

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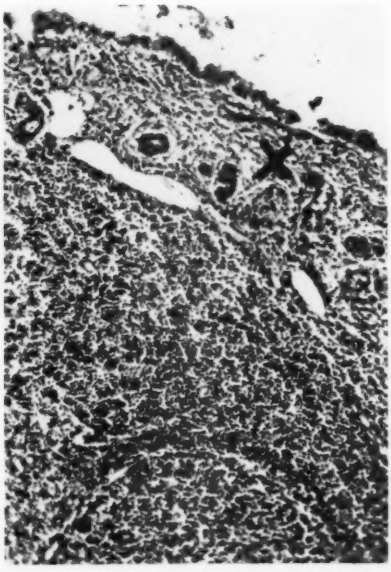

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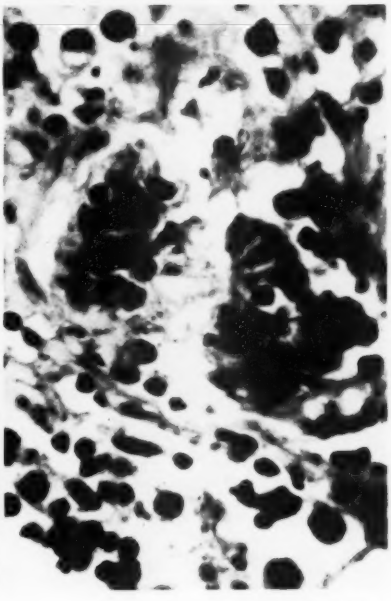


5

Rigdon, Ewing and Tate Effects of Infrared Irradiation

PLATE 58

FIG. 6. Rabbit 1290. Hemorrhages and necroses occur in the area of the brain immediately beneath the region irradiated. Infrared irradiation applied for 5 minutes at a distance of 10 inches. The rabbit was killed 24 hours following irradiation.

FIGS. 7 and 8. Rabbit 1290. A portion of the cerebral cortex unaffected by the infrared irradiation and a similar area of the cortex showing degeneration of the cells and small hemorrhages 24 hours following irradiation. Infrared irradiation was applied for 5 minutes at a distance of 10 inches. Killed 24 hours following irradiation. × 100.

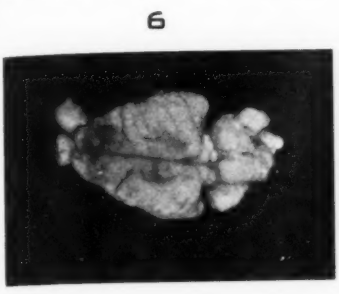
FIG. 9. Rabbit 1291. The periphery of the areas of necrosis in the brain frequently has a zone of leukocytes about it. The skin over the skull was irradiated for 5 minutes at a distance of 10 inches. × 100.

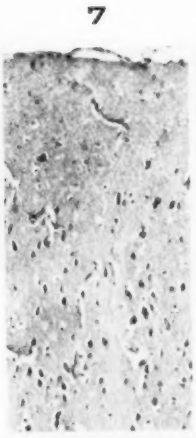
FIG. 10. Rabbit 1234. Focal areas of necrosis may occur in the spleen. Infrared irradiation was applied to the skin over the splenic area at a distance of 10 inches for 5 minutes. Killer 24 hours later.

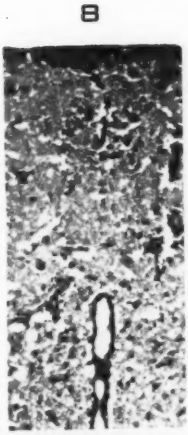
FIG. 11. Rabbit 1234. The lungs are frequently hemorrhagic and congested. Irradiated for 5 minutes at a distance of 10 inches. Killed 24 hours later.

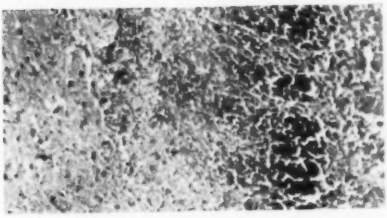
FIG. 12. Rabbit 1234, the same animal as used for Figure 10. In the center of a focal area of necrosis there is a thrombosed artery. It is probable that the necrosis resulted from this vascular occlusion, and that the irradiation produced the vascular lesion. × 36.

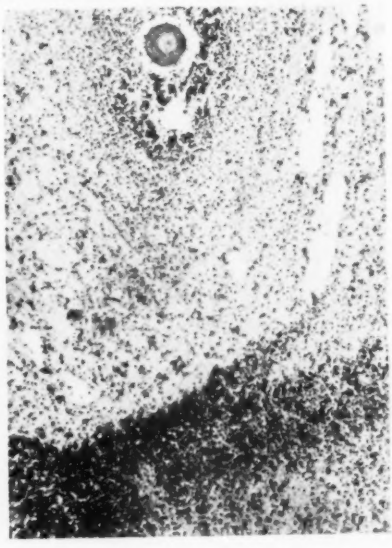
FIG. 13. Rabbit 1242. A focal area of necrosis near the periphery of the liver after the infrared light was placed over this organ. The hepatic cells are severely damaged. Irradiated for 5 minutes at a distance of 10 inches. Killed 24 hours later. × 100.

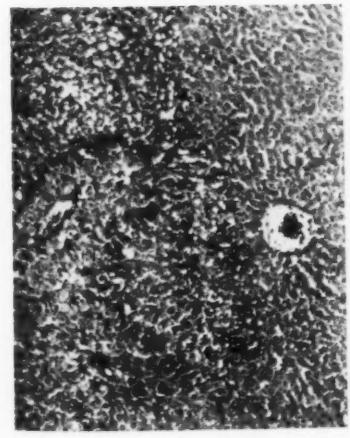












Rigdon, Ewing and Tate Effects of Infrared Irradiation

REPORT OF THE MEETING OF THE COUNCIL

THE AMERICAN ASSOCIATION OF PATHOLOGISTS AND BACTERIOLOGISTS

CLEVELAND

APRIL TENTH, 1943

THE AMERICAN ASSOCIATION OF PATHOLOGISTS AND BACTERIOLOGISTS

Report of the Meeting of the Council
Held at Cleveland, Ohio, April 10, 1943

Present. President CANNON, Doctors FORBUS, GOODPASTURE, HAYTHORN, KARSNER, SOULE, WARREN and WELLER.

The following were elected to membership in the Association:

ERNEST E. AEGERTER
HILDEGARDE ARNOLD
OSCAR AUERBACH
ALICE I. BERNHEIM
ALBERT F. BROWN
CHESTER R. BROWN
FREDERICK I. DESSAU
MARTIN L. DREYFUSS
CHARLES E. DUNLAP
HENRY W. EDMONDS
HUGH A. EDMONDSON
GEORGE L. FITE
A. JAMES FRENCH
ERVING F. GEEVER
ANGELO M. GNASSI
THOMAS A. GONZALES
WILLIAM H. HARRIS, JR.
WEBB HAYMAKER
PETER A. HERBUT
AMBROSE J. HERTZOG
ROBERT C. HORN, JR.
ROBERT S. JASON
HERMAN JOSEPHY
HERMANN LISCO
JOSEPH M. LUBITZ
RICHARD M. MULLIGAN
CHARLES R. REIN
RAYMOND H. RIGDON
WALTER H. SHELDON
DAVID M. SPAIN
SIEGFRIED TANNHAUSER
RALPH M. THOMPSON
PHILIP WASSERMAN
FREDERICK R. WEEDON
MARK C. WHEELOCK

The deaths of the following members were recorded with deep regret:

L. K. BALDAUF
E. A. BAUMGARTNER
WADE H. BROWN

Cancellation of Annual Meeting for 1943. In view of the various problems connected with the war, the Council voted by mail to cancel the annual meeting of 1943.

Since no meeting was held, it was impossible to elect new officers for the ensuing year. The Secretary read Article II of the Constitution as follows: "The business of the Association, including the election of members, shall be conducted by a Council, which shall nominate an-

nually, to be elected by the Society, a President, a Vice President, a Secretary and a Treasurer, to perform the duties usually devolving upon such officers. The same person shall not serve as President more than one year consecutively."

There was extensive discussion of the problem created by the cancellation of the annual meeting. As a result, it was voted that the Council, empowered to conduct the business of the Association, interpret the Constitution to the effect that, as ordinarily provided in more elaborate documents of this sort, the officers shall retain their positions until such time as their successors shall qualify; that this statement be published in the report of the Council in the *American Journal of Pathology,* and that members of the Association who dissent are invited to communicate with the Secretary.

Meeting Place for 1944. Dr. Cannon reported that the invitation of the University of Chicago holds for the meeting in 1944. It was voted to accept gratefully this invitation, provided conditions are such that a meeting can be held in 1944.

Symposium for 1944. It was voted that at the next meeting of this Association, whether in 1944 or subsequently, the topic for the symposium be "Infectious Granulomas, Exclusive of Tuberculosis and Syphilis," and that Dr. Wiley D. Forbus be requested to act as referee. Dr. Forbus accepted this assignment.

Funds of the Congress of American Physicians and Surgeons. The Secretary reported that the funds which had accumulated in the treasury of the Congress of American Physicians and Surgeons had, as a result of the abandonment of the Congress, been turned over to the American Red Cross Society.

Change in Fiscal Year of Association. It was voted to change the fiscal year of the Association to correspond to the calendar year and to the volume year of the *American Journal of Pathology.* Bills to members will be mailed early in January of each year. If payment is not made by March 1, a second notice, indicating delinquency, will be mailed and if payment is not made by April 1, the names of those still delinquent will be removed from the mailing list of the Journal. New members will be billed immediately after election and upon payment they will receive the Journal beginning with the first number of the current year.

HOWARD T. KARSNER, *Secretary*